RELEASE OF CARBON AND NITROGEN FROM ACID PEATS AS INFLUENCED BY SOME TREE SPECIES

A PhD THESIS BY J.M. CAMPBELL.
UNIVERSITY OF EDINBURGH, 1988

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DECLARATION

THIS THESIS HAS BEEN COMPOSED BY ME FROM THE RESULTS OF MY OWN WORK, EXCEPT WHERE OTHERWISE STATED, AND NO PART OF IT HAS BEEN PRESENTED FOR A HIGHER DEGREE.

J.M.CAMPBELL
APRIL, 1988.

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ABSTRACT OF THESIS.

In forested oligotrophic peats in Britain and Ireland, the mineral N released during the decomposition of native organic matter is likely to be the main source of N for plant uptake. On these sites, N release rates appear to be limiting plant growth rates. During decomposition, much microbial metabolite-N is not released directly as mineral N but is diverted into further synthesis reactions and stable N pools. These pools, the products of humification, are comprised in large part of phenolic polymers that have have an affinity for microbially-derived N. The *in vitro* turnover of some phenolic polymers - lignins and humic acids - can be enhanced by a labile carbon supplement. In soils, labile C appears to be a key driving variable for some microbial processes and a postulated consequence of labile C deficiency in oligotrophic peats is a low turnover of N-retaining humus constituents and a correspondingly low N release rate. This thesis investigates if there are variations in labile C availability in peats under different tree species and assesses whether increased labile C availability is related to increased release of mineral N from native organic matter.

In a microcosm experiment involving spruce (*Picea sitchensis* Bong.) Carr), pine (*Pinus contorta* Dougl.)and spruce-pine mixtures grown in a PK-fertilized acid peat, N uptake was 39% higher by pine than by spruce. Substantially greater quantities of soluble carbohydrate were measured in de-rooted peats under pine than in those under spruce. Basal respiration and other microbial attributes - the rate of utilization of a glucose amendment and the mineralization rates of an amino-N amendment were all significantly higher in peats under pine than in those under spruce ($p<0.01$). Soluble carbohydrate levels correlated well with plant uptake rates of N ($r=0.77$; $p<0.01$) and with the mineralization rates of an amino-N amendment ($r=0.79$; $p<0.01$). It is not clear, however, whether the differences reported are related to the tree species themselves or to their mycorrhizal fungi. All de-rooted *planted* peats had substantially more soluble carbohydrate, including free glucose, than *unplanted* controls. Also, mean basal respiration rates, glucose-amendment utilization rates and amino-N amendment mineralization rates were up to 5.5, 3.3 and 4.6 times higher respectively in planted treatments than in unplanted ones.

In a routine sampling programme of a poorly humified *Sphagnum* peat, pH 3.4, under pure and mixed cultures of greenhouse-grown spruce, pine, larch and birch, mean free glucose concentrations in de-rooted samples from birch (333-1642 $\mu g\ g^{-1}$ dw) and spruce-birch (293-701 $\mu g\ g^{-1}$ dw) treatments were consistently higher throughout the

year than in all other treatments, particularly *un*planted controls (86-107 μg g^{-1} dw). Marked seasonal variations in free-glucose levels were recorded, characterised by lowest concentrations in summer and highest in winter-spring and autumn-winter. Free glucose concentrations provided a good index of soluble carbohydrate concentrations (r values>0.97).

Results from a field study indicate that measurable quantities of soluble carbohydrate, including free glucose are present in all decomposing field substrates, including forest FH material and diverse peats. The amounts measured possibly represent the balance between enzymatic release of soluble C from native organic matter and uptake by microorganisms. In most cases soluble C concentrations provide a good indication of decomposition rate. Results from abiotic studies show that mineral N, P, K Ca and Mg supplements have no positive influence on the decomposition rate of an acid oligotrophic peat.

To conclude, this thesis has shown that tree species can take up different amounts of N from the same volume of peat and it has demonstrated significant correlations ($p<0.01$) between soluble C availability and C and N release which can be subject to further enquiry. Experimental procedures developed for use in these studies are described in detail.

ACKNOWLEDGEMENTS

My thanks to John Morgan and Helen Mackay for many helpful discussions and for critically appraising many of the results; to Andrew Gray for support in the laboratory; to David Williams for advice on the statistical analysis of some results; to Bob Muetzelfeldt for making it possible for me to present this thesis using *Macintosh* computing facilities; to my parents for continued encouragement and support; and to the Department of Education (N. Ireland) for funding this research. Special thanks to my supervisor, Douglas Malcolm, for enthuasistically giving advice and support, and for commenting in detail on earlier drafts of the manuscript.

DEPARTMENT OF FORESTRY AND NATURAL RESOURCES
UNIVERSITY OF EDINBURGH. EDINBURGH. EH9 3JU. U.K.

CHAPTER I

(INTRODUCTORY CHAPTER)

FACTORS INFLUENCING THE RELEASE OF CARBON AND NITROGEN FROM REFRACTORY SUBSTRATES.

CHAPTER ABSTRACT.

Most of the N that passes into microbial biomass during the early stages of decomposition is not retained as biomass-N but is diverted into further synthesis reactions. These reactions occur between nitrogenous microbial metabolites and a number of phenolic humus constituents, primarily lignin degradation products and humic substances. Put another way, much microbial metabolite N does not appear to follow a simple degradative pathway towards mineralization but a circuitous one through further synthesis reactions and stable N pools. In high C:N litters, there is often a preponderance of phenolic polymers that have an affinity for microbially derived N and this results in a shift from litter to humus as a source of mineral N for plants. The release rate of this N is determined not just by overall decomposition rates but also by the turnover rates of phenolic humus fractions. Ironically, the decomposition rates of high C:N substrates appear to be commonly limited, not by a low availability of N but by a low availability of C. This conclusion is supported by numerous field fertilization studies and from disparate laboratory decomposition studies of a wide range of refractory substrates, including conifer litter, forest FH material, podzol organic horizons, peats and wheat straw. Laboratory studies also confirm that the *in vitro* degradation of lignins and humic acids can be enhanced by a labile C supplement. Vegetation releases labile C to soils through root exudation, translocation through mycorrhizas and litter inputs. Preliminary investigations have revealed potentially large differences in labile C inputs that can be related to differences in species and in the conditions of growth. This raises the possibility that some species enhance their nitrogen status by increasing the decomposition rate of N-retaining humus fractions. On oligotrophic peats, mixed pine-spruce and larch-spruce forests have a higher N capital than adjacent pure spruce ones. The possibility of a link between labile C inputs to peats and N release rates warrants closer investigation.

THIS THESIS IN A SPECIFIC CONTEXT: FOREST TREE NUTRITION.

Sitka spruce (*Picea sitchensis* (Bong.) Carr) is of major importance to plantation forestry in Britain and Ireland. Although potentially highly productive, in the absence of nitrogen fertilizer, it develops severe N deficiency in heathland soils and deep oligotrophic peats (Weatherell, 1957; McIntosh, 1983). Observations have indicated that under these conditions, the growth of spruce can be considerably enhanced when it is cultivated in mixture with pine or larch (Weatherell, 1957; Zehetmayr, 1960; O'Carroll, 1978; McIntosh, 1983; Carlyle, 1984; Carlyle and Malcolm, 1986a) In these mixed stands, foliar analysis demonstrates a major improvement in foliar N concentrations (O'Carroll, 1978; McIntosh, 1983; Carlyle, 1984). This improvement is also reflected in the foliar N *capital* of mixed stands (O'Carroll, 1978; Carlyle, 1984) and in increased mixture tree biomass (O'Carroll, 1978; Carlyle, 1984).

Effective herbicide control of *Calluna* on experimental plots, indicates that improved mixture growth is *not* related to the potential suppressive effects of *Calluna* on spruce growth (Weatherell, 1953; Robinson, 1972; Malcolm, 1975). Budget analysis on these sites also suggests that increased litter N inputs from mixture stands are quantitatively unimportant with respect to estimated N uptake rates (Carlyle and Malcolm, 1986a). In one of the few sites where adjacent comparable plots of *pure* pine and *pure* spruce plots have been present, Carey *et al.*(1984) have reported that total N capital in pine stands was 50% higher than in spruce stands, despite only 8% difference in root mass.

These differences raise the prospect that N release from native peat is reduced in the presence of some species (e.g. Sitka spruce) *or* that it is enhanced in the presence of others (e.g. pines, larches). Mixed-species planting on oligotrophic peats is now comonplace, but a mechanistic understanding of the mixture *effect* has remained elusive. Several prospective explanations have been proposed. The main ones are:

(1) That evapotranspirational losses related to differences in either rooting architecture or to canopy interception are greater in mixed stands over the growing season, and that as a result of a fall in the water-table, rooting depth increases, allowing greater access to mineral nitrogen.

(3) That (for some reason) extensive mycorrhizal development is inhibited

in pure spruce stands grown on oligotrophic peats.

(4) That microbial saprophytic activities in peats are limited by a shortage of available carbon and that a more lucrative labile-C economy is present in peats under mixtures, associated with pine or larch root exudation and/or pine or larch litter inputs.

In the knowledge that labile carbon has been identified as driving variable for the decomposition of a number of low quality substrates - as discussed in detail later - my own attention to this subject has focussed *firstly*, on the possibility that some species can be associated with more lucrative labile C economies in their underlying peats than others; *and secondly*, on the possibility that species-related inputs of labile C to peats have a positive influence on N release rates.

The questions which set the scene for this research are:

(1) Do different tree species take up different amounts of N from the same volume of peat?

(2) Do some tree species contribute more soluble C to peats than others?

(3) Do levels of soluble C relate to CO_2 release rates? †

(4) Do levels of soluble C relate to mineral-N release rates? ††

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† In this thesis, the term *CO_2 release*, often abbreviated to *C release* is used interchangeably with the terms *C mineralization* and *respiratory activity*, however strictly speaking, the terms are not synonymous. CO_2 (or C) release occurs at a whole-substrate level and it is the variable which is measured here, while C mineralization or respiratory activity occur at a cellular level and are not measured. CO_2 release rates may be influenced by substrate diffusion gradients as well as by respiratory activities. *Under constant conditions both rates could be expected to be identical*, however following sample manipulation (sampling, de-rooting, sub-sampling etc) any such constancy is interrupted by an increase in the diffusion gradient. Subsequently, the residual reserve of substrate CO_2 can be expected to contribute initially to the CO_2 which is released. As discussed later (Chapter 2), routine incubation of subsamples of peat in the studies reported here always involved an initial period of 24 hrs during which CO_2 release was not measured. This eqilibration period was used to allow the conditions of samples - including their CO_2 diffusion gradients - to adjust to the conditions of the incubation *and to allow residual CO_2 to be released.*

†† In this thesis the term *mineral-N release*, often abbreviated to *N release*, is used interchangeably with the term *net N mineralization*, however strictly speaking the terms are not synonymous. Mineral-N (or N) release occurs at a whole-substrate level and is the variable which is measured here while net N mineralization occurs at a molecular level. N release rates may be influenced by the abiotic, chemical release of fixed (non-exchangeable) NH_4^+ as well by net N mineralization .

THIS THESIS IN A GENERAL CONTEXT: RECENT DECOMPOSITION RESEARCH.

Influence of substrate variables on decomposition processes.

Influence of refractory substrate components on N dynamics. I. N accumulation II. N release.

Influence of deficiency factors on the decomposition of refractory substrates. I. Nitrogen. II. Carbon.

Influence of labile C availability on microbial strategies.

Influence of plants on labile C availability.

Influence of substrate variables on decomposition processes.

Though nitrogen is one of the most abundant nutrient elements in forest ecosystems, in northern lattitudes it frequently becomes the most growth-limiting one. This may occur whenever a relatively high quantity of a forest's N capital becomes tied up in refractory plant litters and humus which are slow to decompose and release their nutrients. The chemical composition of a litter is a key determinant of its decomposition rate (Berg and Söderström, 1979; Berg and Staaf, 1981; Melillo *et al.*, 1982; Berg, 1986). Compositional features that have tended to be considered useful indicators of substrate quality or *decomposibility* have been based on the analysis either of *whole-substrate* material, such as initial N concentration (Parr and Papendrick, 1978; Berg and Staaf, 1980; Edmonds, 1980), often expressed in terms of initial C:N ratio; or of *particular substrate fractions*: soluble C or N substances (Knapp *et al.*, 1983; Reinersten *et al.*, 1984; McClaugherty *et al.*, 1985; Jawson and Elliot, 1986) cellulose (Berg *et al.*, 1984) or lignin content (Fogel and Cromack, 1977; Berg and Staaf, 1980; Melillo *et al.*, 1982).

In northern European coniferous ecosystems, low concentrations of soluble substances (Heal and Ineson, 1984) and high concentrations of acid-insoluble aromatic substances, especially lignin (McClaugherty and Berg, 1987), are generally considered to be unfavourable for decomposition. This broad generalization, however, frequently cannot account for observed differences in decomposition rate at a finer scale of variation. There are two main reasons for this: *firstly*, the rate of decomposition of one group of compounds is often dependent on the rate of decomposition of another (McClaugherty and Berg, 1987): in *Sphagnum* peats, for example, the presence of an unusual lignin appears to greatly retard the decomposition of cellulose (Rumjantzewa, 1939; Farmer and Morrison, 1964); *and secondly,* the influence of substrate characteristics on decomposition rate depend on

the time-period of decomposition that is studied (Heal and Ineson, 1984): it is frequently reported, for example, that initial decomposition rates in pine litter are closely related to initial nutrient concentrations, but that subsequently, the effect of nutrients disappear and the influence of lignin becomes prominent (Berg and Staaf, 1980; Berg *et al.*, 1982b; Berg *et al.*, 1987).

Another important and often unacknowledged complication is that the factors which regulate the rates of decomposition of substrates may be different to those which regulate the rates of mineral nitrogen release: it is not necessarily the case, as is sometimes implied, that a faster decomposition rate results in a faster nitrogen release rate (Söderström *et al.*, 1983; McClaugherty *et al.*, 1985; Hendrickson, 1985). Recent information indicates that the potentially positive influence of a fast initial decomposition rate on N release rate can subsequently be overriden by other factors (Bosatta and Staaf, 1982), such as the accumulation of phenolic humus constituents which have a strong retentive power for N (Berg *et al.*, 1987; He *et al.*, 1988). The conditions which determine the timing and rate of mineral-N release are not well understood and new theories and ideas may may be required if a more meaningful insight is to be attained (Berg *et al.*, 1982b).

One old idea that has been a common source of confusion is that there is a *fixed* critical C:N ratio at which substrates release N and that this ratio approximates to the ratio of decomposer biomass (Lutz and Chandler, 1946; Mulder *et al.*, 1969; Alexander, 1977; Packham and Harding, 1982). This has led to a widely promulgated view that microbial utilization rates of high C:N substrates are limited by a deficiency of N, and that the critical C:N ratio represents the transition from an N to a C deficiency, These ideas cannot be upheld, at least in substrates comprised in large part of refractory C fractions (Heal *et al.*, 1982; Berg and Ekbohm, 1983; Heal and Ineson, 1984): in the light of considerable experimental (Meentemeyer, 1978; Aber and Melillo, 1980; Berg and Staaf, 1980, 1981; Berg *et al.*, 1982b; Staaf and Berg, 1982; Flanagan and Van Cleve, 1983; McClaugherty and Berg, 1987) and theoretical (Bosatta and Staaf, 1982; Bosatta and Berendse, 1984; Bosatta and Agren, 1985; Paustian and Schnurer, 1987) evidence, it has become clear that critical C:N ratios can vary considerably, at least between 25 (Upadhyay and Singh, 1985) and 167 (Berg and Staaf; 1981).

More recent ideas have placed particular emphasis on the accumulation of N in phenolic humus constituents rather than biomass as decomposition proceeds; and on

the prospect that the turnover rate of these constituents is intimately related to the release rate of nitrogen.

The nature of the refractory substrate fractions that become key sources for mineral N is not yet clear, however. As discussed later, they may made up of components synthesised extracellularly from reactions of nitrogenous microbial metabolites with modified lignins and humic substances (Nömmik, 1965; Bremner, 1967; Legg *et al.*, 1971; Berg and Staaf, 1980; Berg and Staaf, 1981; Jansson and Persson, 1982; Stevenson, 1982) or of relatively discrete microbial metabolites such as fungal melanins (Ladd and Paul, 1973; McGill *et al.*, 1975; Paul and Juma, 1981; Kelly and Stevenson, 1985; He *et al.*, 1988), often described as *humic acid-like* substances (Bremner, 1967; Huntjens, 1972; Linares and Martin, 1978; Martin and Haider, 1971,1979; Verma and Martin, 1976; Almendros *et al.*, 1985).

Whatever the precise nature of these stable N fractions, the scheme for decomposition which is discussed here, envisages that litter-N becomes increasingly concentrated in phenolic humus fractions rather than biomass as decomposition proceeds; and that an understanding of the conditions which determine the turnover rate of these fractions is important for an understanding of N release.

Influence of refractory substrate components on N dynamics. I. N accumulation.

Prior to N release, there is generally a relative increase (viz. concentration increase) if not an absolute increase of N in substrates as the more labile litter fractions are consumed (Berg and Staaf, 1981; Berg *et al.*, 1987). If an absolute increase occurs, it is usually an outcome of N importation from surrounding material through invading fungal hyphae (Staaf and Berg, 1977; Berg and Söderström, 1979). Where an absolute increase occurs, an important observation is that the amount of N that becomes bound up in fungal biomass during the N acumulation phase is very small in comparison with the amount that becomes bound up in substrate components (Berg and Söderström, 1979; Berg and Staaf, 1981; Berg and Theander, 1984; Berg, 1986).

In the past, it has frequently been assumed that N accumulates in microbial biomass as decomposition proceeds, and that the pool of microbial biomass N subsequently becomes the main source of N for plant uptake. Berg and Staaf (1981), however, have shown that during a period of N accumulation, the increase in the amount of N

in the lignin/humus fraction roughly corresponded to the total amount taken up. This indicates that microbial activity and turnover, at least in the N accumulation phase of decomposition, can be primarily associated with a build-up of N in secondary substrate components rather than in biomass (Bremner, 1967). An indication of the potential rate of accumulation of refractory components can be seen in the results of Almendros *et al.* (1985), who found that after just ten days, humic acid-like (melanins) and fulvic acid-like substances amounted to over 20% of fungal biomass.

The affinity of polyphenolic substances for N has been widely demonstrated (Haider *et al.*, 1965; Ladd and Butler, 1966; Martin and Haider, 1969; Haider and Martin, 1970; Duchaufour and Jacquin, 1975; Nömmik and Vahtras, 1982; Sivapalan, 1982). Indirect evidence for this affinity can be seen in the close association between a plant litter's polyphenol content and the N concentration of its humus (Sivapalan, 1982; Sivapalan *et al.*, 1985). Widespread reports of substantial incorporation of applied fertilizer-N into humus (Foster *et al.*, 1985; Ladd and Amato, 1986; He *et al.*, 1988) may also be related to reactions between humus polyphenols and applied N.

Phenolic units in decomposing substrates are formed both from the degradation of plant polyphenols and from the microbial synthesis of non-aromatic plant carbon (Martin and Haider, 1971; Stevenson, 1982). There are three main classes of polyphenol-rich components in decomposing litters: lignins, fungal melanins and humic substances. These are biochemically ill-defined substances, however. In fractionation procedures, fungal melanins usually register as humic acids (Almendros *et al.*, 1985; He *et al.*, 1988), while the commonly used method for lignin analysis - sulphuric acid or *Klason* lignin - also registers secondary compounds, including humification products† (Berg and Theander, 1984; Kögel and Bochter, 1985; Berg, 1986; Kögel, 1986; Johansson *et al.*, 1986; McClaugherty and Berg, 1987). The increase in the amounts of secondary compounds during humification may explain why it is that measured values for *lignin* often indicate an absolute increase as decomposition proceeds (Berg, 1984; Berg and Theander,1984; Berg *et al.*, 1984; McClaugherty *et al.*, 1985; Berg *et al.*, 1987). There may actually be very little original lignin contained in humus (Felbeck, 1971). Most lignin may be

† An alternative method - the alkaline CuO oxidation - is reported to be suitable for the measurement of lignin in humified materials (Kögel and Bochter, 1985; Kögel, 1986, but unlike the *Klason* lignin method, it has only recently been applied to quantitative studies of litter decomposition (Johansson *et al.*, 1986).

converted to new polymers which include microbial metabolites, and which are more resistant to decomposition than the original lignin molecules (Martin and Haider, 1979). The term lignin, then, has no definite meaning in the context of decomposing substrates, and is used mainly for the sake of simplicity (Berg and Theander, 1984).

Lignin is probably the main source of phenolic units for the formation of humic substances (Haider *et al.*, 1975; Flaig *et al.*, 1975; Stevenson, 1982). This was demonstrated empirically by De Haan (1977), who found that the amount of humus formed over a 10-year period was closely related to the initial lignin content of litters. More direct evidence for the genetic association between lignin and humic substances has been obtained from ^{14}C tracer studies. Martin *et al.*(1974), for example, have shown that the lignin fraction is by far the greatest contributor of ^{14}C to humic acids. Related studies have shown that after just one year, 64% of ^{14}C-labelled lignin could be recovered in the humic acid fraction while only 25% had been respired (Stott *et al.*, 1983).

To reiterate, the regulatory significance of lignin in *nitrogen* dynamics appears to be related to chemical reactions between the phenolic units of lignin or decomposition products and the N derived from the microbial decomposition of other more labile litter fractions (Bremner, 1967; Nömmik and Vahtras, 1982). Probably as a consequence of this, *the strong retentive power of a lignin-rich litter for N lies in its potential for producing a greater quantity of pre-humic substances with an ability to incorporate N* (Aber and Melillo, 1982; Bosatta and Staaf, 1982; Heal *et al.*, 1982).

As indicated, the separation of microbially derived N fractions remains fairly arbitary: studies of chemically partitioned humus fractions have not revealed the precise nature of the phenolic fractions that are most active in the context of N release. Nor is it yet clear whether it will be possible to generalize about the conditions that influence the decomposition of these fractions with respect to the organisms involved, their extracellular enzymes and the conditions that promote their activities.

Influence of refractory substrate components on N dynamics. II. N release.

The microbial release of N from refractory litter usually does not occur until long after decomposition has commenced. This is apparent in north European coniferous forests. In pine litters, with initial N concentrations of around 0.4% (C:N=125), it is

reported that even after 4 years *no* net release occurred, despite a mass loss of c. 70% (Berg *et al.*, 1987).

When the point of N release from coniferous litter is reached, substrates are comprised to a large degree of lignin and humification products, and *once N release commences*, there is now strong evidence that the *rate* of release is proportional to the weight loss of this lignin-humus fraction (Berg and Staaf, 1980; Berg *et al.*, 1982a; Aber and Melillo, 1980; Melillo *et al.*, 1982). In *peats*, the situation is less clear. There is some evidence that under undisturbed, constant moisture conditions, poorly humified peats release little or no N (Morgan in prep; this thesis, chapter 4) in contrast to more highly humified ones (this thesis, chapter 3) and this may indicate that humification and the turnover of humic constituents are also essential for N release from peats.

While the N concentrations of humus components could be expected to provide additional *circumstantial* evidence of their importance to N release, a complicating factor in the measurement of humic-N concentrations is that the results very much depend on extraction and purification procedures (Hobson and Page, 1932; Bremner, 1967): the use of *0.1 M sodium pyrophosphate,* for example, is reported to result in considerable losses of humic-N (Bremner, 1967). Against this background, reported humic-N concentrations need to be considered in the context of the extraction procedures employed. Kononova (1960) suggests that the N concentration of humic acids falls into the range 3.5-4%, while Stevenson (1982) presents estimates that range between 2 and 6%.

Direct evidence for the importance of humified fractions as sources of mineral N has been acquired from ^{15}N tracer studies. These studies indicate that substantial amounts of applied ^{15}N become stabilized in non-biomass humus constituents and and that these may be largely responsible for the N that is released (Legg *et al.*, 1971; Paul and Juma, 1981; Juma and Paul, 1984; Jansson and Persson, 1982; He *et al.*, 1988).

An example of the importance of non-biomass humus material as a *sink* for N can be seen in the study by He *et al.* (1988), for example, who reported that within just one week, a soil which received a composite ^{15}N/glucose amendment had immobilized 50% of applied ^{15}N in the humic and fulvic acid fractions. It was thought that these were comprised to a large degree of fungal melanins. An example of the importance

of non-biomass humic material as a *source* of N can be seen in the study by Juma and Paul (1981), who reported that non-biomass fractions contributed 76% of mineral N, of which more than half came from a stabilized N fraction with a half-life of 27 years, while biomass was a source of only the remaining 24%.

To conclude, the activity and turnover of microbial biomass prior to N release can be primarily associated with the accumulation of N in substrate fractions rather than in biomass. During this phase there is a high level of synthesis activity involving N, as reflected in the transformation of N from a relatively labile state in plant litter to a more refractory state in phenolic substrate fractions (Legg *et al.*, 1971; Jansson and Persson, 1982). Once N release has commenced most N release may occur directly from substrate, through extracellular enzyme activity, rather than indirectly, via microbial biomass. Whether release is direct or indirect , the very close relationship between C release and N release during the decomposition of microbially derived N components (McGill *et al.*, 1975) indicates that *the N release phase of decomposition can be considered to be characterised by a preponderance of degradative reactions*. This contrasts with the N accumulation phase.

Influence of prospective deficiency factors on the decomposition of refractory substrates. I. Nitrogen.

The effects of fertilizer-N and labile-C amendments to substrates in a late phase of decomposition have been widely investigated. Attention to the potential importance of labile carbon as a deficiency factor in *forest soils* has arisen largely out of widespread reports on the failure of N additions to stimulate microbial respiratory activity: the frequency of decreased respiratory activities in FH and O material after ammonium nitrate additions (Tenney and Waksman, 1929; Zottl, 1960; Viro, 1963; Fessenden *et al.*, 1971; Nömmik and Popovic, 1971; Williams, 1972; De Jong *et al.*, 1974; Roberge, 1976; Kowalenko *et al.*, 1978; Bååth *et al.*, 1981; Söderström *et al.*, 1983; Hendrickson, 1985) and ammonium sulphate additions (Salonius, 1972; Foster *et al.*, 1980) indicate that *low N concentrations* (or high C:N ratios) *in organic soils do not necesarily give rise to N-limited decomposition.* This conclusion has also been reached from much longer-term studies on a wide range of podzolized forest soils (e.g. Bååth *et al.*,1981; Söderström et al., 1983) which have shown that soil microbial activity, measured both by respirometry and fluorescence microscopy, was still significantly lower than control plots 3-5 years after *urea* fertilization. Bååth *et al.* (1981) found that the decrease in activity was proportional to the amount of N applied.

Frequently *urea* treatment of an acid soil induces a initial positive respiratory response (e.g. Salonius, 1972; Foster *et al.*, 1980; Söderström *et al.*, 1983). This is often assumed to be a positive effect of NH_4-N produced by urea hydrolysis. An alternative explanation that has received much experimental support, however, is that the pH increase results in increased solubility of C compounds, which in turn provide the substrate for increased microbial activity (Jackman, 1960; Salonius, 1972; Ogner, 1972; Foster *et al.*, 1980, 1985).

Circumstantial evidence for the idea that the positive impact of urea on respiration is related to pH rather than to N was reported by Salonius (1972), who found that in LFH material from a spruce forest, respiratory responses to applications of urea, KOH alone, and KOH in combination with ammonium sulphate, were primarily related to the change in pH. The presence of mineral-N actually reduced the positive effects of a pH increase on respiration. From these results, it was concluded that microbial activity was enhanced by the increased availability of carbon rather than by the addition of available nitrogen. Direct evidence for urea-induced solubilisation of soil C was reported by Salonius and Mahendrappa (1975). Foster *et al.* (1985) looked at the effects of urea on C solubilisation in detail, and showed that incremental increases in urea amendment of forest LF material, resulting in pH increases of up to 2.7 units, induced corresponding increases in the amounts of extractable C by up to 10 times the amount in unamended material.

One possible explanation for the observations of longer-term reductions in microbial activity following urea fertilization (e.g Bååth *et al.*, 1981; Söderström *et al.*, 1983) is that initial solubilization of carbon after fertilization leads to a subsequent depletion of labile C reserves. This was demonstrated by Ogner (1972), who found that urea fertilization subsequently led to a 30% reduction in the carbohydrate concentration of a raw humus.

There are two explanations for why *mineral-N* additions may *reduce* the decomposition rates of substrates: *firstly,* the addition of mineral salts can greatly reduce substrate pH levels (this thesis, chapter 6) and this in turn may reduce carbon availability; *secondly,* there is now evidence that NH_4, even in low concentrations will reduce the decomposition rate of lignins, by repressing the ligninolytic system (Keyser *et al.*, 1978; Kirk *et al.*, 1978; Kirk and Fenn, 1982).

As indicated at the outset, these N fertilization studies have led to support for the idea that substrates with high C:N ratios can be limited by a shortage of readily available C rather than a shortage of available N. This conclusion has also been reached from plant microcosm research (Bååth *et al.*, 1978; Clarholm, 1985; Fisher and Gosz, 1986b); litter (Harmer and Alexander, 1986) and wheat straw (Knapp *et al.*, 1983; Reinersten *et al.*, 1984) decomposition experiments; and from *in vitro* lignin (Ander and Erikkson, 1975; Kirk *et al.*, 1976; Kirk *et al.*, 1978; Keyser *et al.*, 1978) and humic acid (Mishustin and Nikitin, 1961; Myrsha, 1966; Szegi, 1966) degradation studies as discussed below.

Influence of prospective deficiency factors on the decomposition of refractory substrates. II. Carbon.

Even under the most favourable experimental conditions investigated, the decomposition of lignins (Kirk *et al.*, 1978) and humic substances (Mishustin and Nikitin, 1961) is a slow process. This may indicate that the main reason for the relcalcitrance of these components is that their complex, heterogeneous structures confer mechanical or chemical resistance to enzymatic attack (Stevenson, 1982). It is clear, however, that some fungal species require labile carbon supplements in order to degrade lignin (Kirk *et al.*, 1976; Kirk *et al.*, 1978; Keyser *et al.*, 1978; Fenn and Kirk, 1981) and that others which *can* degrade lignin in the absence of labile carbon show higher rates of degradative activity in the presence of a supplement (Ander and Eriksson, 1975). A similar situation appears to be common to humic substances, which in the absence of a glucose supplement undergo very little (Mishustin and Nikitin, 1961; Myrsha, 1966) or no (Szegi, 1967) degradation. This suggests that the low amount of energy stored in the these substances (Kirk and Fenn, 1981) is a further reason for their recalcitrance.

The mechanistic nature of *the priming effect* of labile C on lignin and humic acid degradation is not known. It is probable that enhanced enzymatic activity has to be preceded by initial growth and that the breakdown of aromatic rings in lignins and humic substances proceeds too slowly to support initial growth (Keyser *et al.*, 1978; Kirk and Fenn, 1982).

Influence of labile C availability on microbial strategies.

Existing concepts pertaining to microbial growth strategies can usefully complement other guiding ideas in decomposition research. Here, attention is called to some ideas about *microbial strategies* as a means of addressing an interpretative problem

concerning the role of C additions on the decomposition of soil organic matter.

It is clear that substrate quality deteriorates as decomposition proceeds and the more labile litter fractions are consumed. There is also evidence that the changes in quality are accompanied by changes in the decomposer microflora and that the explicit taxonomic changes which are commonly observed (Hayes, 1965a,b) are underlain by implicit functional ones. In particular, there is much support for the idea that high initial substrate quality favours microbial species which are capable of explosive population growth rates and rapid exploitation of labile substrate fractions - the *r strategists* - while deteriorating quality favours species which are capable of more sustained population growth on the more refractory substrate residues - the *k strategists* (Heal and Ineson, 1984). Other related changes between taxonomic and functional characteristics that might occur as substrate quality deteriorates are an increase in those microorganisms which have a capacity for the production of antibiotics (the penicillia) and in those with a more diverse accoutrement of enzymes (the basidiomycetes) (Swift, 1976).

While these somewhat simplified generalisations need to be assessed more critically if they are to be used as the basis for a general ecological theory (see Swift, 1976; Bowen, 1980; Pugh, 1980; Heal and Ineson, 1984), they are sufficient in this instance to illustrate a potential problem associated with C amendment studies. It is conceivable that a labile C input favours microorganisms which have a capacity for rapid biomass accumulation and rapid exploitation of labile nutrients, but which contribute negligibly (if not negatively) to the release of N from phenolic humus constituents. For this reason, increased CO_2 release in response to a labile C supplement cannot be assumed to indicate enhanced recycling rates of nitrogen. This interpretative dilemma is especially relevant to rhizosphere-C studies, where enhanced soil respiratory activities in the presence of plant roots provide no information about the functional attributes of the microflora or of their contribution to the recycling of nutrients.

Influence of plants on labile C availability.

In the knowledge that labile C can act as a driving variable for lignin and humus decomposition, it is pertinent to consider whether vegetation might affect the decomposition of soil organic matter by influencing labile C availability. The two main ways that vegetation could effect the labile C economies in underlying organic matter are:

(1) by adding labile carbohydrate to soils through through litter inputs or through live roots: directly, as exudates, or indirectly as secondary metabolites associated with mycorrhizal fungi.

(2) by modifying soil conditions in a way that favours increased solubilisation of labile C from native soil organic matter by microbial enzymes.

Tracer experiments using ^{14}C have provided information on the amounts of organic matter exuded by plant roots and on the plant and environmental factors that influence these amounts. Measured concentrations of exudate probably represent a summation of C secreted as mucilage and C released by lysis of tissue at the root surface (Martin, 1977a, b).

Exudates are largely comprised of low molecular weight C compounds. These can be readily separated into three main fractions: carbohydrates, mainly present as mono- and disaccharides; organic acids, especially acetic acid; and a wide range of amino acids. The amounts and composition of exudate can vary considerably (Hale and Moore, 1979), however, according for example, to species (Smith, 1969; Smith, 1976) and genotypes (Kipe-Nolt *et al.*, 1985); the age of plants and plant roots (Smith, 1970; Hamlen *et al.*, 1972; Reid and Mexal, 1977); the extent of root abrasion (Ayers and Thornton, 1968); oxygen availability (Grineva, 1962; Ayers and Thornton, 1968; Reid and Mexal, 1977), water stress (Reid, 1974; Martin, 1977b; Reid and Mexal, 1977); and the presence of microorganisms (Ivanov *et al.*, 1967; Rovira and Ridge, 1973 Barber and Lynch, 1977; Martin, 1977a; Vancura *et al.*, 1977; Martin and Kemp, 1986), including nitrogen-fixing bacteria (Lee and Gaskins, 1982) in the rhizosphere.

While mycorrhizal development appears to greatly increase translocation of photosynthate to the roots (Harley and Lewis, 1969; Ho and Trappe, 1973; Reid *et al.*, 1983), it is not clear how this alters the pattern of exudation.

Most studies of exudation have been confined to young plants, particularly cereals, cultivated in axenic culture in order to prevent transformations and losses of exudates by rhizosphere microorganisms, but in the absence of microbial activities at the root interface, the amounts measured seem likely to be much less than would occur in the field (Barber and Martin, 1976; Barber and Lynch, 1977; Martin, 1977a; Vancura *et al.*, 1977). Even in axenic culture losses can be considerable, however. Martin (1977a), for example, found that 28% of the ^{14}C

photosynthate transferred to the roots of wheat plants grown in sterile soil could be recovered in a soil hydrolysate.

In field studies, conducted over a period of a few weeks or more, it is not possible to separate *root exudates* from *readily decomposable root fractions* as sources of C in the rhizosphere. A field experiment which looked at the combined input of these fractions to soil was reported by Kieth *et al.*(1986). This study involved a series of ^{14}C labelling events throughout the growing season of a wheat crop followed by destructive sampling and measurement of ^{14}C in plant roots and soil 3 weeks after each labelling event. From the results, it was calculated that 25% of the 1305 kg C ha^{-1} that was transported to the roots over the growing season was present in de-rooted soil. The actual input of readily-decomposible C to soils was probably much higher, for these values exclude the contribution of microbes to respiratory losses during the 3-week periods between labelling and sampling. Inputs on this scale could be expected to have a profound impact on microbial processes.

Studies which have been conducted on *tree root* exudation include those by Slankis et al.(1964), Smith (1969), Reid (1974), Reid and Mexal (1977), Smith (1976) and Reid *et al.*(1983). In a comparative study of exudation of axenically cultivated seedlings representing a range of tree species, Smith (1969) found that the aptly named Sugar pine (*Pinus lambertiana*) , for example, exuded very much more than the three other species of pine used in the study.

In an investigation of exudation in 7-year-old lodgepole pine (*Pinus contorta* Dougl), Reid and Mexal (1977) found that more than 40% of the ^{14}C transferred to roots could be recovered in the root bathing solution, and when *actively exuding roots* only were considered, the amounts of ^{14}C lost to solution sometimes increased to more than 80%.

Using procedures for the collection of exudates from mature trees, Smith (1976) attempted to compare several different species on a forest-plot basis and found substantial variations. While in view of the vagaries of plot-related conditions, it would be premature to ascribe these differences to species *per se*, potentially very large differences between species have been highlighted that could be subject to further investigation.

There are probably large variations in the amounts of labile C in the litters of

different tree species. Amounts depend on mass of litter as well as on the concentrations of labile C *in* the litter (Spalding, 1977). In the British uplands needle retention times for Scots pine and Sitka spruce have been estimated to be 2.3 and 6.7 years, respectively (Innes et al., 1986), and this could be the main factor determining the quantitative input of labile C in litter.

In the early stages of decomposition, the quantity of soluble C measured probably represents a combination of residual C at the start of senescence as well as release by plant cell enzymes *during* senescence. As shown later (chapter 5), microbial cellulolytic activity is probably responsible for the presence of a small soluble C reserve in substrates after *decomposition* has commenced. Circumstantial evidence for the influence of microbial enzymes is the presence of soluble carbohydrate in well-decomposed litters, forest FH layers and peats (chapters 3, 4 and 5), while more direct evidence can be seen in the *increases* in the amounts of soluble carbohydrate in bracken petioles one year after incubation in the field (Frankland, 1969). These reserves of soluble C probably represent a balance between enzymatic release from non-soluble substrate components and microbial uptake.

Irrespective of the relative importance of exudates and litter as sources of labile C on soil microbial activities, it appears that the effects of labile C inputs are usually superimposed on other plant influences on the soil microflora. Shields and Paul (1973), Jenkinson (1977) and Fisher and Gosz (1986) for example, all suggested that the main impact of plants on decomposition rates was related to the depletion of soil moisture levels.

This highlights a general problem with field studies: the effects of soluble carbohydrate inputs on decomposition processes cannot be separated from the effects of soil microclimatic changes induced by plants. Microcosm studies, in contrast, permit greater control of soil microclimate and offer some scope therefore for isolating other plant factors that might account for modified decomposition rates.

Two microcosm studies have recently been undertaken, one with wheat plants (Clarholm, 1985) and the other with Douglas fir (*Pseudotsuga menziesii* Franco) seedlings (Fisher and Gosz, 1986b). Both studies concluded that plants had a positive influence on microbial activity, as measured by fluoresence microscopy and respirometry respectively, and both suggested that this was due to inputs of

readily available C from plant roots. Whereas Clarholm found that plants greatly increased the decomposition of soil organic matter, as evidenced in the increased amounts of N in the fraction *inorganic N plus soil organism and plant N,* the results of Fisher and Gosz were less clear-cut: although they found that N release rates were greatly modified by plants, they concluded that the rates both increased and decreased depending on the time-interval investigated and that the overall change was not significant.

Notably, Clarholm found that the addition of glucose to microcosms with or without plants, increased the transfer rate of N from soil to biomass. This was taken to be supportive evidence for the view that *the positive influence of plants on the decomposition of native soil N is related to the release of C from plant roots.*

CHAPTER 2.

(METHODOLOGICAL STUDIES)

INTEGRATED PROCEDURES FOR THE ANALYSIS OF SOLUBLE CARBON, MINERAL NUTRIENTS, AND C AND N RELEASE DURING DECOMPOSITION.

INTRODUCTION.

In decomposition research there is a need for procedural improvements to enable many facets of decomposition to be studied concurrently. At present, at least two extractants are commonly employed for the extraction of mineral nutrients, and where low molecular weight organic compounds are also being measured, a third extractant is often considered necessary. Where respirometric and incubation studies are involved, the number of sub-samples required is further increased. If *root-free* samples of rhizosphere soil are needed for these studies, the absence of an integrated procedure can impose severe constraints on the scale and scope of an investigation, not least because the de-rooting of samples is a tedious and time-consuming process. For the purposes of this research programme, it was considered important to have access to rapid, routine procedures that would allow large-scale multivariate investigations to be undertaken without undue demands on operator time. Priority was given to developing an integrated approach that would allow for: (1) the use of *small samples* of de-rooted material for all analyses, including CO_2-release measurements; (2) the *simultaneous extraction* of mineral nitrogen and soluble carbohydrate; and (3) *automated measurement* of carbohydrate and/or free glucose in addition to other nutrients. The scheme outlined below appears to have been largely successful in meeting these aims, and has also permitted the simultaneous extraction of PO_4-P, K, Ca and Mg in addition to mineral N and soluble carbohydrate.

Preparation of samples for analysis.

Three sets of sub-samples *only* are required in order to measure levels of soluble carbohydrate, free glucose, NH_4-N, NO_3-N, PO_4, K, Ca and Mg *before* incubations; CO_2 release *during* incubations; and soluble carbohydrate, free glucose, NH_4-N, NO_3-N (and PO_4, K, Ca and Mg if required) *at the end of* incubations.

The first set of sub-samples is taken to determine the dry weights of samples. These are measured after drying at 85°C for 24 hrs. The fresh weight equivalent of 1.5 g dw is then calculated for each sample.

The second set of sub-samples is used to determine initial levels of components. Each sub-sample with the fw equivalent of 1.5 g dw, is placed in a 250 ml volumetric flask *or* a 250 ml winchester bottle, and 2% acetic acid is added to bring the volume of extractant up to 150 ml. The exact quantity of acetic acid required ±0.5 ml is calculated from: *150 - no. ml moisture in sub-sample.* These sub-samples are extracted over-night (c. 16 hrs) as indicated later.

The third set of sub-samples is used to determine CO_2 release during incubations and the levels of mineral N and soluble carbohydrate at the end of incubations. This set is prepared along with the *second* set: the fw equivalent of 1.5 g dw of each sample is placed in a 250 ml winchester bottle. Where substrate quality variations are to be studied independently of field conditions then the moisture levels of all sub-samples need to be increased to a *fixed* level. Moisture levels can be increased with a fine mist-spray. In the studies reported here, samples were brought up to a moisture level of 84%. This fixed level was chosen because (a) in nearly all cases it was higher than the highest *in situ* moisture level and (b) preliminary investigations on a range of FH and peat samples indicated that *for 1.5g dw sub-samples* it was generally close to the optimum for CO_2 release. This set of sub-samples is *loosely* capped and left for 24 hrs at 20°C to equilibrate to the new moisture and temperature conditions and to release residual CO_2that has accumulated in samples during the period of storage in sealed bags. Bottles are then sealed and incubated, as described later.

Use of 2% acetic acid for the simultaneous extraction of soluble carbohydrate, NH_4-N, NO_3-N, PO_4, K, Ca and Mg.

Background. *Acetic acid* has long been used for the extraction of exchangeable metallic cations in carbonate-free soils (Williams, 1928). Acetic acid (3% v/v) in combination with sodium acetate (10% w/v), known as *Morgan's* reagent is also a well-established extractant for PO_4-P in agricultural advisory work in Britain (Allen, *et al.*, 1974), but on account of its high sodium content, it is not well suited for the analysis of other cations by atomic absorption or flame photometry. Recently, acetic acid *alone*

has been compared to a number of other extractants, including 0.25M H_2SO_4 and 1M NH_4Cl, for the extraction of PO_4-P from acid peats (Waughman,1980) and has been found to be equally effective. Potassium or sodium salts have remained the favoured extractants for the extraction of mineral-N.

Recent work in this laboratory, aimed at finding an effective and convenient extractant for soluble carbohydrate, compared 2% acetic acid with a number of other extractants: 1M potassium chloride; 1M sodium acetate; 2% EDTA; 1M tri-sodium citrate; 1M citric acid; and water. 2% acetic acid was found to be superior for two main reasons: *firstly*, because it extracted lower quantities of humic components than the other extractants of higher pH; *and secondly*, because none of the other extractants extracted more free glucose (measured using a glucose oxidase-peroxidase method) than acetic acid. Notably, two of the other extractants (tri-sodium citrate; citric acid) were unsuitable for use in the assay procedure employed for soluble carbohydrate analysis (page 21) because they interfered in the reaction.

In the knowledge that acetic acid is already an established extractant for metallic cations in non-calcareous soils (Allen *et al.*, 1974), a study was undertaken to see if it might also ~~might~~ be suitable for the extraction of NH_4-N. *The relative strength of adsorption of cations on organic matter - $H>Ca>Mg>NH_4\geq K>Na$* - (Lutz and Chandler, 1946; Stevenson, 1982) *suggested that this should be the case.* Using a diverse range of organic substrates - forest litters, FH layers, and peats of varying degrees of humification - this study established that a 2 hour extraction in *2% acetic acid* was comparable for the extraction of NH_4-N as a 2 hour extraction in *1N KCl*. The comparison encompassed a broad range of NH_4-N concentrations, equivalent to 7-1260 $\mu g\ N\ g^{-1}$ dw peat, as shown below:

	1N KCl	2% CH_3COOH	
(1) Amorphous Calluna peat, pH 3.6	7	8	
(2) Fibrous Sphagnum peat, pH 3.4	36	34	
(3) FH layer under lodgepole pine	71	68	
(4) FH layer under Sitka spruce	117	123	
(5) As (3), but after 60-day incubation	558	576	
(6) As (4), but after 60-day incubation	1260	1278	$\mu g\ N\ g^{-1}$ dw peat

This study also confirmed that acetic acid is comparable to ammonium acetate and hydrochloric acid for the extraction of PO_4-P and metallic cations. Morgan (PhD Thesis in prep.) presents the results of a detailed comparative study which support these findings.

Procedures. Samples in 2% (v/v) acetic acid are extracted overnight (16 hrs)* in an orbital shaker and are then filtered through Whatman No 42 filter paper. If levels of soluble carbohydrate, mineral N, P and metallic cations are all to be measured then at least 60 ml

extract is retained for analysis. [*N.B.While inorganic components are extracted over a 2-hour period, maximum extraction of soluble carbohydrate requires an *overnight* extraction].

Comments. 2% acetic acid prepared from *Analar* stock is a convenient, inexpensive extractant which should greatly enhance integrated studies dealing with the role of labile carbon in mineral nutrient dynamics. It is not possible here to provide a mechanistic interpretation of the role of acetic acid in the extraction of inorganic cations. It is not clear, for example, how important exchange reactions involving extractant hydrogen ions are in relation to other extractant characteristics. ***Here, as elsewhere in the literature, the use of acetic acid for the extraction of cations is justified on the basis of an empirical study showing that the amounts of cations extracted by acetic acid correspond to the amounts extracted by neutral salts.***

Routine measurement of soluble carbohydrate and free glucose in soil extracts. I. *Soluble carbohydrate.*

Background. Water- or ethanol-soluble carbohydrate concentrations are commonly measured in litter samples during the early stages in the decomposition of *leaf litter*. Using $Ba(OH)_2$ and water, respectively, as extractants, Jenkinson (1968) and Verstraeten *et al.* (1970), have measured soluble carbohydrate levels in a wide range of *soils* and have reported close positive relationships between the amounts of carbohydrates in extracts and the amounts of N mineralized during laboratory incubations.

Preliminary investigations, carried out as part of this research, suggested that measurable amounts of soluble carbohydrate can be extracted from all organic substrates, irrespective of their state of decomposition. This raised the possibility that the amount of carbohydrate extracted might provide an index of substrate quality (as measured by CO_2 release) and that such an index might sensitively reflect the influence of of plants and abiotic perturbations (e.g. pH changes, nutrient additions etc.) on quality.

Soluble carbohydrate levels are commonly measured using procedures based on a colour reaction involving a concentrated H_2SO_4-*anthrone* reagent. The amounts measured do *not* represent *total* soluble carbohydrate - for which there is no single analytical procedure - because different sugars react at different rates and to different colour intensities with anthrone (Stevenson, 1982), while only one sugar (usually glucose) is generally employed as a standard. Put more specifically, the amounts measured more accurately represent the concentration of *hexose sugars and their polymers* in extracts since hexose sugars contribute primarily to colour development. Here, as elsewhere (e.g. Doutre *et al.*, 1978) however, the term *soluble carbohydrate* is used for the sake of simplicity.

A number of interpretative and procedural problems exist in relation to the use of anthrone for soluble carbohydrate analysis. The reaction involves heating samples in 18 to 26 N H_2SO_4 to between 85 and 100°C (the acid concentration and temperature depending on the method adopted). The main *interpretative* problems are (1) that under such intense hydrolytic conditions dissolved humic substances may release carbohydrate that hitherto has not been neither soluble nor available in a soil sample and that the impact of such release remains an unknown quantity, and (2) that many inorganic components are known to interfere in the reaction (Ivarson and Sowden, 1962; Greenland and Oades, 1975; Doutre *et al.*, 1978) and that the amount of interference again remains an unknown quantity. The main *procedural* problems are (1) that large replicate variation is reported, which relates at least in part to the procedural stage of adding conc H_2SO_4-anthrone reagent to aqueous samples (Doutre *et al.*, 1978), and (2) it is difficult to envisage the procedures being accessible to routine, safe automation.

The small modifications to existing procedures described below, were employed in order (a) to avoid the procedural stage of adding concentrated H_2SO_4 to wet samples; (b) to allow very low concentrations of carbohydrate to be measured (to c. 1ppm); and (c) to permit large numbers of samples to be analysed routinely.

Procedures. Stock anthrone reagent is made up by dissolving 0.50 g anthrone in 1litre of 24N H_2SO_4 which is stored at 2°C. Stock *glucose* standard solutions of 100 and 1000 $\mu g\ ml^{-1}$ 2% acetic acid are maintained and from these, fresh standards of appropriate strength are prepared in 2% acetic acid. 3ml aliquots of 2% acetic acid extracts and standards are added to 25ml boiling tubes. About four replicates of each standard are made up. Samples are then left overnight to dry at 65°C in a fan-assisted oven. The following day, provided that all samples are dry, 4 ml aliquots of cold anthrone reagent are added to each to each tube. Tubes are then placed in a water-bath at 85°C for 15 minutes; every 4-5 minutes they are briefly removed and gently shaken. They are then placed in a bath of cold water. After 5 minutes, absorbance is read in 1 cm cuvettes in a spectrophotometer set at 620 nm. Colour remains stable for about 2 hrs.

Comments. Using these procedures, it is possible for one operator to readily measure carbohydrate levels in up to c. 120 samples per day (2 sets of c. 60 samples). In preliminary studies, using *water* extracts which had been dried overnight, the large replicate variation often noted by others was eliminated: variation

was under 5%. When *acetic acid* extracts were used, however, replicate variation was much greater, up to c. 20%. Overnight drying appears to have resulted in some destruction of glucose. In further preliminary studies aimed at improving the method by minimising the destruction of glucose, the pH of acetic acid extracts was increased in increments up to pH 12 prior to drying, using hydroxides of K, Na and Ca. No improvement was noted, however, and at pH levels over pH 7, apparent destruction was greatly increased.

Despite the problems associated with replicate variation, the correlations between soluble carbohydrate and *free glucose* concentrations (measured enzymatically; see next section) were usually very close, with r values mostly in excess of 0.90 (chapters 3, 4 and 5). This was largely due to the considerable range in soluble carbohydrate and free glucose levels usually encountered in comparative studies involving a range of treatments.

Routine measurement of soluble carbohydrate and free glucose in soil extracts. II. *Free glucose.*

Background. D-glucose, occurring free or in polymers is the predominant sugar in soils (Cheshire and Mundie, 1965; Gupta, 1967; Greenland and Oades, 1975; Doutre *et al.*, 1978; Stevenson, 1982). It is also a sugar that can readily be associated with the synthetic activities of plants rather than those of microorganisms, and it is generally reported to be quantitatively the most important carbohydrate measured in root exudates. In recent years it has been common to use glucose-induced respiration for the estimation of microbial biomass in soils (Anderson and Domsch, 1978) and to use glucose amendments as a means of assessing the potential importance of labile C in decomposition and nutrient release dynamics. A rapid and sensitive procedure for the measurement of glucose could contribute in a number of ways, therefore, to an asessment of the chemical and biological properties of a substrate. Despite the drawbacks of the anthrone procedure (as outlined above), it continues to be a widely employed procedure for the measurement of glucose in glucose amendment studies. I report below on a rapid, simple and sensitive procedure for the determination of glucose in soil extracts. This can be applied directly to the measurement of glucose in acetic acid extracts and can be readily modified for use with other extractants.

Procedures. The procedures are based on those described by Trinder (1969) for the determination of glucose in blood. They have been modified (a) in order to make

them suitable for the measurement of the much lower concentrations of glucose often found in soil extracts (viz. to c. 0.5 $\mu g\ ml^{-1}$), and (b) in order to make them suitable for automated use with 2% (0.34M) acetic acid extracts. The procedures have also been simplified (employing one reagent instead of two) and developed with a view to minimising costs.

A stock solution is prepared containing 0.4 M K_2HPO_4 and 0.25 M KOH. As indicated below, when this is combined in a 1:1 ratio with 2% acetic acid, it will give a solution mixture of pH 7.4. This pH is close to optimal for the reaction. In preliminary studies, the colour reaction was found to rapidly deteriorate below pH 6.8, while at the upper end of the pH scale, no significant deterioration occurred until pH exceeded 9.2.

The reagent mixture is prepared as follows: to each 100 ml of the above solution is added:

4	**mg**	**peroxidase**
10	**mg**	**glucose oxidase**
40	**mg**	**4-amino phenazone**
100	**mg**	**phenol**

The phenol should be weighed out and dissolved separately. The reagent mixture is stirred for several minutes until the last traces of 4-amino phenazone have dissolved. About 100 ml reagent mixture is sufficient for c. 40 samples. The reagent mixture will remain stable *in the dark* at 2-4°C for up to 3 days provided care has been taken to ensure that all glassware used in its preparation has been thoroughly cleaned.

Stock glucose standard solutions of 100 and 1000 $\mu g\ ml^{-1}$ 2% acetic acid are maintained. From these, fresh standards of appropriate strength are prepared in 2% acetic acid. Stock standard solutions can be retained for at least 3 months.

Automated analysis is performed as follows: the reagent mixture is combined with samples in a 1:1 v/v ratio. In the studies described here, *reagent*, *sample* and *air* tubes with a flow rate of 0.8 ml min^{-1}were used. An acetic acid wash was used at a flow rate of 1.2 ml min^{-1} or more. The wash time was 2.1 mins and the sample time 0.4 mins. The sampling rate was therefore 24 samples h^{-1} and at this rate the cost was c. *1 UK £ per 30 samples.*

The sample-reagent mixture passes through a coil retained in a water-bath at 37°C and

then into a 10 or 20 mm flow cell. Using a 20 mm cell a glucose concentration of 5 μg ml^{-1} gave an absorbance reading of c. 0.10. Background (*i.e.* auto-)absorbance of samples was measured using a reagent mixture which had no enzymes. In most cases, autoabsorbance levels were generally negligible, and routine measurements were considered unnecessary. Only samples containing noticeably high concentrations of dissolved humic matter gave significant autoabsorbance readings which therefore had to be subject to routine measurement.

Glucose present in the fraction *free glucose plus soluble polymers of glucose* can also be measured using this assay procedure, but it is first necessary to hydrolyse the polymers. This can be done as follows: 10 ml aliquots of soil extracts and glucose standards are injected into *Macartney* bottles along with 5 ml aliquots of 1.5 N HCl (giving a net HCl concentration of 0.5 N). Bottles are then sealed with caps which have been fitted with *silicon rubber* seals and autoclaved at 121°C for 1 hour (Autoclaving times up to 4 hrs were tested, but no further increase in glucose levels were measured). The *mineral* acid is then neutralised using 5 ml aliquots of 1.5 N KOH. Stock solutions of 1.5 N HCl and 1.5 N KOH are always tested beforehand to ensure that a 1:1 ratio of the two gives a pH approaching neutrality (a pH in the region of pH 5 to pH 9 is adequate).

Comments. Other saccharides do not interfere in the reaction. A number of cations - NH_4, Na, K, Ca and Mg - and anions - NO_3, PO_4, Cl - were tested for interference, but none was found. These procedures can be readily adapted for manual use, as follows: 2 ml aliquots of reagent mixture are added to 2ml samples of extract in 10 ml test-tubes. Tubes are gently shaken and then placed in a water-bath at 37°C for 15 minutes. They are then transferred to a bath of cold water. Absorbance is read in a spectrophotometer at 520 nm.

Where neutral extractants are employed, instead of acetic acid, then a 0.1 M K_2HPO_4/KH_2PO_4 buffer solution should be used in place of the K_2HPO_4/KOH solution mixture described here.

Procedures for the measurement of CO_2 release during incubation.

Background. Numerous procedures are available for the measurement of CO_2 release rates from soil (*for review*, see Anderson, 1982). Laboratory methods

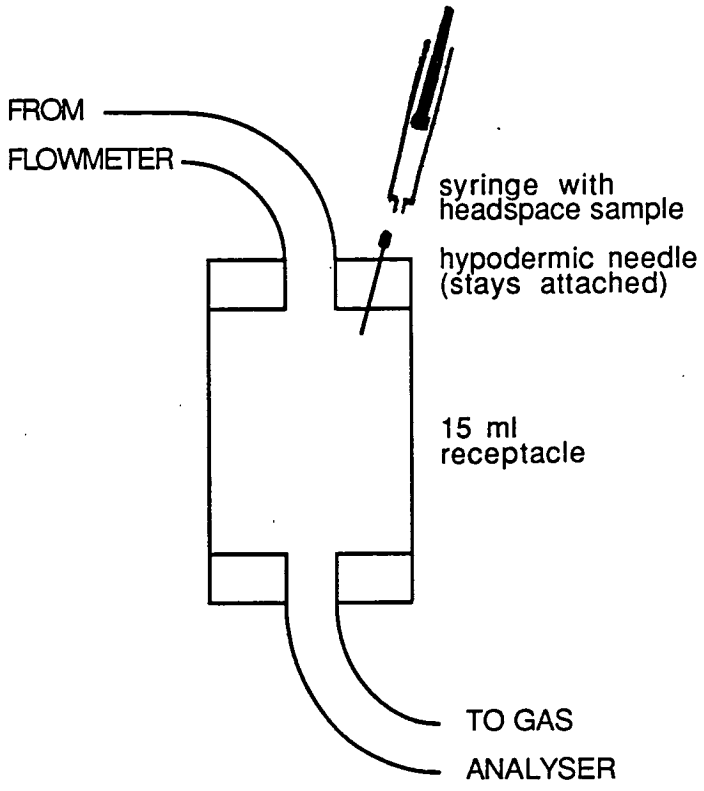
involving the passage of CO_2-free through a sample chamber (soil column or respiration vessel) and into an IRGA usually make large demands both on IRGA-time and on operator-time, though automated valve systems (Brooks and Paul, 1987; Hendricks *et al.*, 1987) have recently increased the number of analyses which can be performed (c. 30 measurements h^{-1}). Procedures based on the sampling of the headspace of a respiration chamber of known volume with a syringe (e.g. Sparling, 1981; Sparling *et al.*, 1981) have recently been widely employed, however measurement of CO_2 concentrations has invariably been carried out by gas chromatography. I report below on the use of IRGA for the measurement of CO_2 concentrations. IRGA permits a very high rate of sampling (≥100 injections h^{-1} as opposed to 30-40 for g.c.) and in this research has been found to have a sensitivity in excess of that reported for g.c. (0.2 μg CO_2 as opposed to 3.8 μg CO_2; Van Cleve *et al.*, 1979).

Procedures I. As noted earlier, samples which have been prepared for incubations are left to equilibrate for 24 hrs at 20°C in *loosely capped* 250 ml winchester bottles. [The volume of bottles should be determined beforehand: for the purposes of calculations, it is best if all bottles have the fixed volume ± 2%]. Bottles are then tightly sealed using 28 mm R3 open screw-caps which contain 2 X 25mm dia. 3mm silicon rubber discs (Esco Rubber, Sterilin, U.K.). At regular intervals during an incubation, the concentration of CO_2 in the headspace is sampled as described below.

A constant stream of CO_2-free air, obtained using a soda-lime filter, is pumped through plastic tubing and into the *absolute-mode* receptor an IRGA. A receptacle for receiving head-space samples, c.15 ml volume, is positioned in this air-stream c. 1 m or less from the CO_2 analyser. Flow-rates can be adjusted using a flow-meter. In the studies reported here, flow-rates of c. 0.5-1.5 l min^{-1} in plastic tubes of diameter 5 mm were used, however flow-rates and tube-diameters can be varied to suit requirements. The volume of the receptacle and the volume of the tube leading to the CO_2 analyser need to be adjusted to ensure that the sample is not delivered so fast that the system is sensitive to the speed at which the user injects samples into the receptacle, or too slow that the sample is over-diluted in the air-stream and the resolution is reduced.

This system has a resolution down to 0.2μg CO_2 per injection, well in excess of that

reported for gas chromatographs (c.3.8 μg CO_2; Van Cleve *et al.*, 1979) and titrimetric analysis of KOH which has been used to absorb CO_2, as reported below.



The headspace in winchester bottles is sampled using a 1 or 2 ml syringe. Disposable plastic syringes were used in these studies, and with repeatability generally less than 1 or 2%, were found to be satisfactory. In this research a *top* CO_2 gas standard of 1.5 to 2 % (v/v) was prepared in a gas cylinder by introducing CO_2 to a pressure of c. 1.5 atm and then *filling up* with compressed air to a presure of 80-100 atm. The CO_2 concentration of this gas mix was then determined using gas mixing pumps (Wösthoff, West Germany). These operate from a gas cylinder of pure $C0_2$ mixing CO_2 by volume, and have an absolute accuracy of << 1%. On each sampling occasion, further CO_2 standards of lower concentration were readily generated to suit sample CO_2 concentrations using a gas diluter (ADC GD-600).

When all the bottles have been sampled, the screw-caps and silicon rubber discs are removed and after several hours are replaced with loose caps. They are then left overnight and the following day are re-sealed.

The process of ventilating samples can be speeded up by placing uncapped bottles in a fume-cupboard for 1-2 hrs, however this can result in moisture losses from samples leading to some surface-drying. For this reason, over-night ventilation in loosely capped bottles was the preferred method of ventilating in most of the studies reported here. The ventilated headspace of randomly selected samples should be tested in the early stages of an investigation to ensure that the ventilation conditions enable head-space CO_2 levels to return to ambient levels by the time bottles are re-sealed.

The question arises as to how much CO_2 is lost from samples during the period of ventilation. It should not always be assumed that these can be calculated by extrapolation of release rates prior to ventilation. Two potential sources of reduction in release rates during a sampling period are: (1) reduced diffusion gradients as CO_2 accumulates in headspace; and (2) microbial inhibition by high CO_2 concentrations (Macfadyen, 1973). The combined effect of these sources of variation can be assessed as follows: 12 replicate subsamples are incubated in winchester bottles at constant temperature. One set of 3 subsamples is not subjected to ventilation, but is allowed to accumulate CO_2, while 3 other sets are sealed consecutively for only short intervals during the incubation and are left loosely-capped for the remainder of the time. CO_2 release rates in the *no-ventilation* treatment during these intervals is calculated by deduction of the amounts of CO_2 in headspace between consecutive measurements. By comparison of release rates over a period of days/weeks is it possible to determine the maximum concentration of CO_2 which can be tolerated in headspace, before measurements need be taken and a ventilation interval allowed. For most of the work described here an upper limit of 1.5 % CO_2 (v/v) was found to be satisfactory. There was some evidence that release rates in samples of *litter* were not significantly reduced until much higher headspace concentrations were reached (≥3% CO_2), while release rates from *peats* showed small reductions at lower headspace concentrations. *Concentrations up to 1.5% were tested for their effect on mineral-N release rates but none was found.*

Procedures II. A further modification to procedures was developed which allows CO_2 release from samples to occur into CO_2-free air. These procedures are particularly suited to studies of *in situ* CO_2 release from soils and can also be used to

test the effect of CO_2-free air on CO_2 release rates. They can be used to replace procedures based on titrimetric analysis of KOH and offer considerably enhanced resolution.

The procedures are as follow: a sample cup containing 5 ml 1 N KOH is suspended in a sealed vessel containing a soil core or soil subsample in order to absorb the CO_2 which is released. The volume of this vessel is not important for the purposes of calculating CO_2 release rates, however, in preliminary studies its headspace should be sampled using the procedures described above to ensure that all CO_2 which is released is absorbed in KOH. After a known time-interval, the KOH is removed and transferred to a winchester bottle of known volume, and this is then sealed using silicon rubber discs and an open screw-cap. 5 ml 2 N HCl is injected into the bottle through the silicon rubber discs, using a stainless steel hypodermic needle. The bottle is then placed on a rotary shaker for 10 minutes and a headspace gas sample is taken as before *(Procedures I)*.

The solubility of CO_2 is a function of solution pH and the partial pressure of CO_2 in the gaseous phase (i.e. headspace). To put it more precisely, however, the amount of CO_2 present in solution as CO_3^{2-} and HCO_3^- is influenced by pH, and in mineral acid can be seen from pK values to be infinitely small:

$$H_2CO_3 \equiv\equiv\equiv HCO_3^- + H^+ \qquad pK_a = 6.4$$

$$HCO_3^- \equiv\equiv\equiv CO_3^{2-} + H^+ \qquad pK_a = 10.4$$

At low pH, then, all dissolved carbonate is present as H_2CO_3. Under these conditions, the amount of CO_2 in solution (all as H_2CO_3) can readily be calculated , simply from a knowledge of the partial pressure of CO_2 in headspace:

$$CO_{2\ (g)} \equiv\equiv\equiv H_2CO_3^{\circ}{}_{(soln)} \qquad \log K^{\circ} = -1.46$$

or $$[H_2CO_3] = 10^{-1.46}\ p\ CO_{2\ (g)}$$

or $$\log\ [H_2CO_3] = -1.46 + \log p\ CO_{2\,(g)}$$

where p is the partial pressure expressed in atmospheres. Hence from a knowledge of CO_2 in headspace it is possible to calculate the amount of carbonate in solution. Provided the headspace volume is much greater than the solution volume (c.>20

times), it can be calculated that virtually all CO_2 is present in headspace and that the amount present in acidified KOH (as H_2CO_3) is not significant (*see worked example below*). If the amount of CO_2 calculated to be present in acid is considered to be significant, however, it can be added on to the amount of CO_2 measured in headspace. Where computer interfacing facilities are available, any such corrections can be carried out automatically since they are solely a function of measured headspace CO_2 levels. In the studies described here, such corrections were not necessary.

This combined acidification-headspace assay procedure takes about the same time as a titrimetric assay procedure and offers greatly enhanced resolution: its resolution of 0.2 μg CO_2 compares with a reported value of 44 μg CO_2 for titrimetric procedures Van Cleve *et al.*, 1979).

Comments. These procedures can be employed for a wide range of experimental investigations. With minimum demand on operator time (up to 100 sample injections per hour) up to several hundred samples can easily be handled in any single investigation. The procedures have been employed in a large number of studies, including: the determination of optimum moisture levels for CO_2 release in different substrates; the effects of pH changes on CO_2 release from peats; the effects of labile carbon and mineral nutrients (singly and in combination) on release rates; comparative studies of litters and soils associated with different tree species on CO_2 release.

Worked example. Consider a winchester bottle containing 10 ml of acidified KOH, with a total volume of 260 ml, and a headspace volume therefore of 250 ml. Its headspace CO_2 concentration is measured as 2.0 % v/v (i.e. p CO_2 =0.02 atm). At s.t.p. the amount of CO_2 still remaining in solution after acidification is therefore:

$$[H_2CO_3] = \text{antilog}(-1.46 + \log 0.02)$$
$$= 0.694 \text{ mmoles } CO_2\ l^{-1}$$
$$= 6.94 \text{ μmoles } CO_2 \text{ } 10ml^{-1} \text{ acidified KOH}$$
$$\equiv 0.155 \text{ ml } CO_2 \quad \textit{(from 1 mmole } CO_2 \textit{ =22.4 ml)}$$

With a headspace of 250 ml and a concentration of 2.0 % CO_2, the *proportion* of CO_2 remaining in solution is:

$0.117 \div (250 \times 0.02) = 0.031$ (i.e. 3.1%)

After acidification, therefore, 97% of carbonate is released into headspace. The amount of CO_2 which was originally absorbed by alkali can therefore be calculated to have been:

$250 \times 0.02 \times (100 \div 97) = 5.15$ ml CO_2

CHAPTER 3

(MICROCOSM STUDY)

EFFECTS OF SPRUCE AND PINE ROOTS ON MICROBIAL ACTIVITIES IN AN OLIGOTROPHIC PEAT: A MICROCOSM STUDY.

CHAPTER ABSTRACT.

The effect of vegetation on the decomposition of soil organic matter has not yet been clearly resolved. Positive and negative impacts of plant roots on microbial processes have been reported, but the overall implications of these for different soils or different plants are unclear. Previous research indicates that on oligotrophic peats, N uptake by Sitka spruce is considerably enhanced in the presence of pine or larch. The increased litter inputs in such mixed plantations appear unable to account for the increased N uptake. The prospect that greater mineralization of native peat-N is occurring under mixed stands than under adjacent pure ones has therefore been contemplated. This report describes a microcosm study designed to show whether there are differences in microbial activity or N release under lodgepole pine and Sitka spruce. With PK fertilization, N uptake was 39% higher in pure pine culture than in pure spruce culture. Basal respiration and other microbial attributes - the rate of utilization of a glucose amendment and the rate of mineralization of an organic-N amendment - were all significantly higher in de-rooted peats under pine than under spruce ($p<0.01$). All planted peats had considerably higher levels of soluble carbohydrate (including free glucose), respiratory activity, glucose utilization and amino-N mineralization than corresponding unplanted controls, however there is no evidence to indicate higher levels of peat-N mineralization in the presence of plants: if N release rates are reflected in N uptake values, then results could indicate that there has been a suppressive effect on N release from native organic matter by spruce rather than an enhancement effect by pine. In the absence of detailed information on mycorrhizal characteristics and the impact these may have on saprophytic N release processes, it cannot be assumed that the apparent species-related differences reported here are likely to persist in other situations.

INTRODUCTION.

In forested oligotrophic peats in Britain and Ireland, subjected to the standard forestry practice of phosphorus and potassium fertilisation, low levels of mineral nitrogen appear to limit the growth of Sitka spruce (*Picea sitchensis* (Bong.) Carr) (McIntosh,1981). In contrast, many other species - pines, larches, birches - show no marked signs of growth check or N deficiency under similar conditions.

Frequently, enhanced growth of Sitka spruce cultivated in mixture with pine or larch has been reported (Weatherell,1957; Zehetmayr,1960; O'Carroll,1978; Carlyle,1984) and on several experimental sites, recent research has established that substantially greater N uptake occurs in mixed spruce stands than in adjacent pure spruce ones (Carlyle,1984). Budget analysis also indicates that litter-N inputs at these sites are quantitatively unimportant with respect to N uptake rates (Carlyle,1984). While the main driving mechanism accounting for uptake differences remains elusive, it appears that any explanation for the *mixture effect* must invoke greater mineralization of native peat-N under mixed spruce stands than under adjacent pure spruce ones. This raises the prospect that some species (*e.g.* pines, larches) influence mineralization rates more than others (*e.g.* Sitka spruce).

Little definite information has emerged about the factors controlling mineralization processes in forest soils. Recent attention has focussed increasingly on the potential importance of *lignin* as a carrier for N and on the apparent link between lignin decomposition rate and N release rate (Berg and Staaf,1980; Berg *et al.*,1982b; Aber and Melillo, 1982).

Two main forms of nutrient amendment have been applied to recalcitrant substrates in an effort to enhance their decomposition rates: mineral N and labile C. The widespread failure of mineral-N additions to stimulate microbial activity (Tenney and Waksman, 1929; Zottl,1960; Viro,1963; Fessenden *et al.*,1971; Nömmik and Popovic,1971; Salonius,1972; Williams, 1972; Foster *et al.*, 1980; Bååth *et al.*,1981; Söderström *et al*, 1983; Hendrickson, 1985) suggests, however, that N availability is not a pervading constraint during the decomposition of acid wastes, despite high C:N ratios. Recently, the presence of ammonium and amino nitrogen has actually been implicated in a repression of fungal enzyme systems and in associated reduced decomposition rates (Keyser *et al.*, 1978; Kirk *et al.*, 1978; Fenn and Kirk, 1981; Kirk and Fenn, 1982; Berg *et al.*, 1982a). In contrast, labile C amendments have generally been found to enhance microbial activity in low quality substrates (Kirk *et al.*, 1976; Kirk *et al.*,1978;

Bååth *et al.*, 1978; Foster *et al.*,1980; Sparling *et al.*, 1981; Flanagan and Van Cleve, 1983; Heng and Goh, 1984) though a positive impact on *nitrogen release* is only occasionally reported (Clarholm,1985; Harmer and Alexander,1986).

Unfortunately, a clear understanding of the impact of an amendment on decomposition is frequently precluded by the different times-scales operating between the two main decomposition processes measured - net C mineralization (a widely used index of overall microbial activity) and net N mineralization - and by the absence of any general relationship between them over an arbitary time interval : the short term respiratory response typically apparent following a C amendment, gives no clue to the potentially much longer term repercussions for N release.

The effect of plant roots on the decomposition of soil organic matter has not yet been clearly resolved and could involve positive effects, induced by exudation of labile carbon (Jager, 1971; Coleman, 1976; Clarholm, 1985) or moisture uptake; or negative effects, induced by increased competition with microbes for mineral nutrients, *excessive* moisture uptake (Babel, 1977; Fisher and Gosz,1986a) or production of allelopathic compounds by roots or their mycorrhizal fungi (Gadgil and Gadgil, 1971,1975; Sparling *et al.*, 1982).

In order to study the impact of plant roots on decomposition processes under conditions free from the potential swamping effects of soil moisture fluctuations (from transpiration), seasonality, or litter inputs (seasonal or otherwise), it is generally desirable to move away from field situations to the more controlled environments of a greenhouse or growth chamber.

Two such *microcosm* studies have recently been undertaken. The first, reported by Clarholm (1985), was set up with wheat plants to look at the role of *root-derived carbon* in soil N mineralization, while the second, using Douglas fir seedlings (Fisher and Gosz, 1986b), was designed to look more generally at the impact of *plant roots* on N mineralization.

Clarholm found that plants substantially increased the level of *inorganic-N plus biomass-N* (plants; microbes; protozoans) with respect to unplanted controls and showed that even greater increases could be obtained using glucose amendments instead of plants. The results of Fisher and Gosz were less clear-cut: while demonstrating that plants had a major impact on net N mineralization, they found that the effect was both

positive and negative depending on the time interval studied during the course of the experiment. Notably, they also found that de-rooted soil obtained at harvesting evolved considerably more CO_2 than unplanted soil, an outcome which they attributed to labile C inputs from plant roots.

This report describes the results of a microcosm experiment designed as part of a wider study looking at potential differences in the effects of species and species-mixtures on the decomposition of recalcitrant organic substrates. The primary aim of the experiment was to assess whether the *roots of different species* - as opposed to their *litters* - differ in their capacity to influence microbial activity and net N mineralization. A secondary aim was to provide an appropriate experimental setting for looking at some factors that might account for observed differences. The experiment involved two species, Sitka spruce (*Picea sitchensis* Bong.) Carr), here abbreviated to SS, and lodgepole pine (*Pinus contorta* Dougl.), here abbreviated to LP.

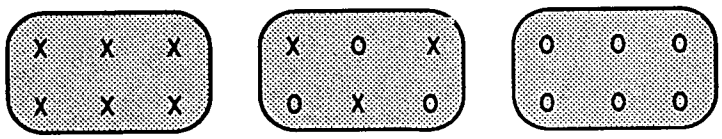
METHODS.

The experimental design comprised six treatments, each with five replicates:

Treatment	1	2	3	4	5	6
Fertilization	0		PK			NPK
Species	SS	SSLP	SS	LP	SSLP	SS

The experiment aimed to look mainly at differences between the *PK-fertilized* treatments: treatments 3, 4 and 5. Treatments 1, 2 and 6 were included for reference purposes.

Each replicate consisted of a plastic tub, 27 X 15 X 8 cm, containing 320 g dw peat packed to a dry wt. density of 0.75 g cm^{-3}, and six seedlings. In the case of mixed-species treatments, tubs contained three seedlings of each species planted alternately as shown :



Planting arrangement for pure and mixed cultures of spruce(X) and pine(O).

Plant spacing was equivalent to 250 plants m^{-2} .

The peat was a moderately humified mixed *Sphagnum-Calluna* peat, pH 3.5, obtained from commercial sources operating on a nearby lowland raised bog. It had been milled, and was sieved through a 4 mm screen before use. Chemical analysis is referred to later (Table 6a).

Seedlings were one year old at planting. They were selected for size to minimise variation within and between species. Thirty-two individual seedlings from each species were used to obtain initial values for dry wt. and nutrient content (N, P, K, Ca, Mg).

Fertilizer additions were made at planting, and on two subsequent occasions after random tests indicated soil concentrations nearing depletion. Nitrogen was added in solution as NH_4NO_3; phosphorus and potassium as a solution mixture of K_2HPO_4 and KCl. N, P, and K were applied in the ratio 100 : 18 : 66. The levels of initial application, shown below, were based mainly on reported values for nutrient uptake in Sitka spruce seedlings (Benzian and Smith, 1973) :

No.weeks after planting	0	52	75	Total
N	150	100	50	300
P	27	18	9	54
K	99	66	33	198

Mineral nutrient applications during the experiment (mg per plant).

Conditions in the growth room during the experiment were as follows : light was supplied at a rate of 250-350 µmoles m^{-2} sec^{-1} over a 16 hr day; temperature was maintained at 20°C, RH at 80%. Tubs were watered with distilled H_2O in order to keep soil moisture levels within the range 70-80%; every two weeks they were weighed and brought up to 80% moisture. To synchronize a late phase of bud breaking, tubs were removed from the growth room after c. 42 weeks and placed in the dark at 4°C for 6 weeks. Litter was removed every few weeks and stored for subsequent analysis. The experiment was run for 90 weeks (21 mths).

The sampling format at the end of the experiment was as follows: tubs were removed at random over a period of several weeks and plants excised at the root collar. By careful excavation, as much as possible of individual root systems was then recovered. Most of

the broken fine root that remained - which always amounted to less than 20% of the total root weight in each tub - was removed after sieving through a 6mm mesh. Finally, a sub-sample of c. 30 g dw was taken from the homogenised peat that passed through the sieve, and any remaining fine root fragments were removed with forceps and weighed. The sample of *de-rooted* peat remaining was then stored for up to 3 weeks at 2°C and used in the assay procedures described below.

Plant samples were dried at 85°C for 48 hrs. Shoot, needle and root weights were measured for each plant. The weight of broken fine root in each tub was apportioned among the six individual plants according to the relative weights of their intact root systems. For each tub, composite samples of the shoots, needles, roots and litter of each species were prepared for chemical analysis by grinding through a 0.5 mm screen.

Analyses of the de-rooted peat samples obtained from planted treatments were as follows: the fresh weight equivalent of 1.5 g dw of each sample was extracted overnight in 150 ml of 2% acetic acid and filtered through Whatman No 42 filter paper. Three other 1.5g subsamples were prepared and used in incubation, glucose utilization and amino-N mineralization studies as described:

Incubations were carried out as follows: subsamples were containerized in 250 ml winchester bottles and moisture levels increased to 84% of fw using a fine mist spray. Bottles were loosely capped and allowed to equilibrate overnight at 20°C. They were then sealed using two 4mm silicon rubber discs (Esco Rubber Co.,UK) held in place by 28mm open screw caps. Subsequent headspace CO_2 measurement and ventilation procedures were as described previously (this thesis, page 26).

Glucose utilization was measured as follows: subsamples were left to equilibrate overnight at 20°C and then combined with 1.5 ml of a glucose solution containing 2500 μg glucose ml^{-1}. Moisture levels were adjusted to 84% with a fine mist spray and subsamples were stirred manually with a plastic stirrer. They were incubated for 16 hrs at 20°C and then extracted overnight in 2% acetic acid. Unamended control samples were also prepared to ensure that changes in residual peat glucose concentration were quantitatively unimportant with respect to utilization rates of glucose amendments. Glucose levels in extracts were measured as described below

Amino-N mineralization was measured as follows: the fw equivalent of 1.5 g dw subsamples were left to equilibrate overnight at 20°C and then combined with 1.5ml of a solution mixture containing 400 μg ml^{-1} of each of the following: DL-Alanine, L-Arginine, DL-Asparagine, and DL- Aspartic acid. Moisture levels

were adjusted to 84% using a fine mist spray and samples mixed manually using a plastic stirrer. They were incubated for 24 hrs at 20°C and then extracted overnight in 2% acetic acid. Unamended control samples were also prepared to ensure that residual N release rates were quantitatively unimportant with respect to N release from amino acid amendments. NH_4 levels in extracts were measured as described below.

Measurement of extractable levels of NH_4-N, PO_4-P, K, Ca and Mg in soil extracts was carried out as follows. NH_4-N was measured by flow injection analysis and gas diffusion in a Tecator Fiastar 5020 Analyzer: the reaction is based on the diffusion of NH_4 from a stream of NaOH through a PTFE membrane into a stream of indicator solution (Tecator 5000-0295). P (as PO_4) was measured by flow injection analysis in a Tecator Fiastar 5020 Analyzer: the reaction is based on the formation of molybdate blue by reduction with acidic stannous chloride. The sum of NO_3-N and NO_2-N was measured by flow injection analysis in a Tecator Fiastar 5020 Analyzer: the reaction is based on the formation of a diazo dye with acidic sulphanilamide after passage through a cadmium reductor; and a colour reaction with N-(1-Naphtyl)-ethylenediamine dihydrochloride. K, Ca and Mg were measured by atomic absorption/emission using a Pye Unicom Sp 9 Atomic Absorption Spectrophotometer. Residual levels of soluble carbohydrate in de-rooted peat samples were measured using anthrone (this thesis, page 21) and levels of free glucose using a glucose oxidase-peroxidase enzyme assay (Trinder, 1969) developed for automated use with soil extracts (this thesis, page 23).

Total N, P, K, Ca and Mg concentration in plant and peat samples were determined using a modified micro-kjeldahl digest (Allen *et al.*, 1974): two replicate 0.1 g subsamples of each sample were accurately weighed into a pyrex digest tube; 2 ml 36 N H_2SO_4 was added to each tube, followed by 1 ml (dropwise) 30% H_2O_2; tubes were placed on a heating block at 340° C for 5 hrs, and after cooling samples were transferred to 50 ml volumetric flasks and made up to 50 ml with distilled water. N (as NH_4), P (as PO_4), K, Ca and Mg were measured as described above for peat extracts.

RESULTS.

Plants: Plant biomass; N concentration, N uptake; P and K uptake; N:P:K ratios. ***Peats:*** changes in peat Ca and Mg concentrations; residual free glucose and soluble carbohydrate concentrations; glucose utilization over 16 hrs; amino acid mineralization over 24 hrs; C mineralization during incubations; N mineralization and C:N mineralization ratios during incubations; impact of fine root contamination on results.

Statistical Note: as noted earlier, the experiment was designed mainly to look at differences between *the three PK-fertilized treatments*.. The differences in nutrient uptake and biomass changes in these treatments were analysed using the *T'-Method* (Sokal and Rohlf, 1981) and in tables 1-4, different initials in superscript denote significant treatment differences at $p<0.05$. Tabulated results for the no-fertilizer and the NPK-fertilizer treatments are given mainly for reference purposes: treatment differences associated with fertilization regimes were *not* analysed using the *T'Method.*

Plant biomass.

Plant dry matter increased by between 700% (*unfertilized SS*) and over 4000% (*NPK SS*) during the experiment. *Without fertilization* SS biomass production was substantially lower than LP production whether grown in pure or mixed culture (Table 1A, columns 1 and 2). More SS biomass was located below ground than above ground, while the reverse was the case for LP (Table 1B, columns 1 and 2).

Table 1. (A) Mean *increase* in plant biomass of species in each treatment *(g plant^{-1})*; (B) Ratio of *root* biomass:*shoot* biomass; (C) Root biomass at harvesting *(g plant^{-1}). Std. errors in italics. Also see Statistical Note, above.*

Fertilization		0		PK			NPK
Species		SS	SSLP	SS	LP	SSLP	SS
(A)	SS	4.25 *.17*	3.87 *.20*	^{a}8.62 *.30*	- - -	^{a}8.23 *.57*	24.4 *1.1*
	LP	- - -	7.27 *.99*	- - -	^{b}12.1 *0.6*	^{ab}10.0 *1.3*	- - -
(B)	SS	1.08	1.08	^{b}1.22	- - -	^{ab}1.13	0.75
	LP	- - -	0.75	- - -	^{a}0.96	^{a}0.96	- - -
(C)	SS	2.48 *.10*	2.37 *.17*	^{a}4.98 *.24*	- - -	^{a}4.60 *.56*	10.0 *1.0*
	LP	- - -	3.08 *.17*	- - -	^{a}5.56 *.25*	^{a}4.40 *.60*	- - -

With *PK fertilization* LP again greatly out-performed SS, with *40%* more biomass (Table 1A, columns 3 *vs.* 4; Figure 1), though its growth was reduced in the presence of SS (columns 4 *vs.* 5). As in the unfertilized plots, SS apportioned a greater *proportion* of its biomass below ground, in contrast to LP (Table 1B, columns 3 to 5).

The *weight of root* present in *PK-fertilized* treatments showed no species-related trend: in pure culture LP had 13% more root than SS, while in mixed culture it had 4% less (Table 1C).

N concentrations.

At harvesting, N concentrations in needles ranged from 0.44 to 2.52%. *Mean* values are shown in *Table 2*.

Table 2. N concentrations in needles, shoots, roots and whole-plants (%). *Std. errors in italics.*

*Also see **Statistical Note**, page 39.*

Fertilization		0		PK			NPK
Species		SS	SSLP	SS	LP	SSLP	SS
Needles	SS	2.11 *.08*	2.08 *.14*	0.50 *.02*	----	0.50 *.03*	2.01 *.07*
	LP	----	0.99 *.05*	----	0.63 *.03*	0.67 *.07*	----
Shoots	SS	0.94 *.03*	0.86 *.05*	0.28 *.01*	----	0.27 *.01*	0.72 *.04*
	LP	----	0.68 *.05*	----	0.40 *.01*	0.44 *.01*	----
Roots	SS	0.95 *.02*	0.82 *.03*	0.57 *.02*	----	0.47 *.04*	1.12 *.04*
	LP	----	1.01 *.03*	----	0.50 *.02*	0.50 *.03*	----
Whole	SS	1.30 *.01*	1.18 *.05*	[ab]0.50 *.01*	----	[a]0.45 *.02*	1.27 *.04*
plant	LP	----	0.97 *.03*	----	[b]0.54 *.02*	[b]0.55 *.01*	----

The highest N concentrations in needles, shoots and roots were consistently associated with the unfertilized treatments. PK fertilization, while enhancing biomass production induced a substantial dilution in N concentration. This can be shown strikingly by looking at concentration in terms of *N efficiency (mg dw mg-^{1}N)* over the lifespan of the experiment (Table 3B). These values show that under conditions of PK fertilization both species increase in biomass by about the same extent per unit N uptake (columns 3-5), while in the absence of fertilizer, LP puts on more biomass per unit N uptake than SS (columns 1-2).

N uptake.

There was no difference in N uptake between the two *unfertilized* treatments (Table 3). With *PK fertilization,* this level of uptake was sustained for LP, but the presence of SS either in pure or mixed culture resulted in substantially reduced uptake.

Table 3. (a) N uptake per plant (mg) and per growth tub (mg). (B) N efficiency (mg biomass mg^{-1} N). ***Standard errors in italics.***

Also see Statistical Note, page 39.

Fertilization		0		PK			NPK
Species		SS	SSLP	SS	LP	SSLP	SS
(A) Plant^{-1}	SS	56.2	45.3	40.3	-----	32.6	310
	LP	-----	64.0	-----	55.8	49.0	-----
Growth tub^{-1}		337_{15}	328_{10}	$^{a}241_{14}$	$^{b}335_{19}$	$^{a}245_{36}$	1860_{27}
(B)	SS	$76.0_{1.2}$	$84.7_{4.3}$	$^{a}214_{5}$	-----	$^{b}255_{13}$	$79_{2.6}$
	LP	-----	$114_{4.0}$	-----	$^{ab}217_{9}$	$^{ab}222_{56}$	-----

Put into another context, despite up to twice the root weight in PK-fertilized treatments than in unfertilized ones (Table 1C), no more N was taken up, and the presence of SS was actually associated with a substantial reduction in uptake. The results are shown graphically in *Figure 2.*

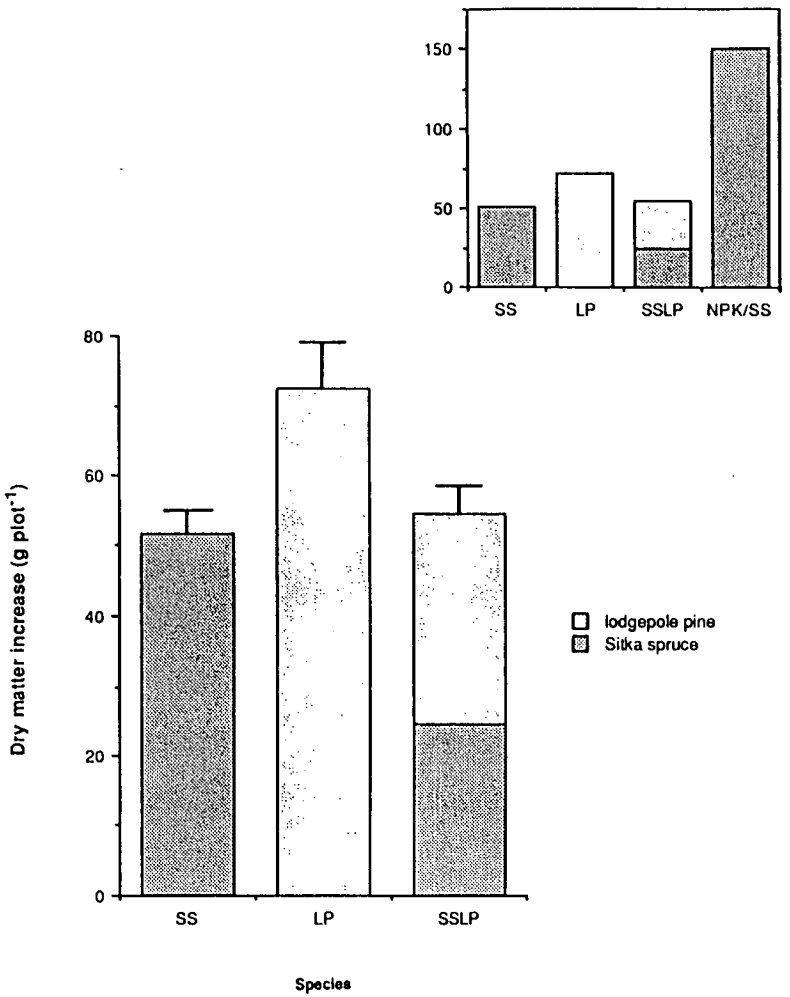


Figure 1. Mean increase in plant dry matter in PK-fertilized plots *vs* species-composition of plots. Inset shows relationship of these PK treatments to the NPK one. Bars show 95% confidence intervals.

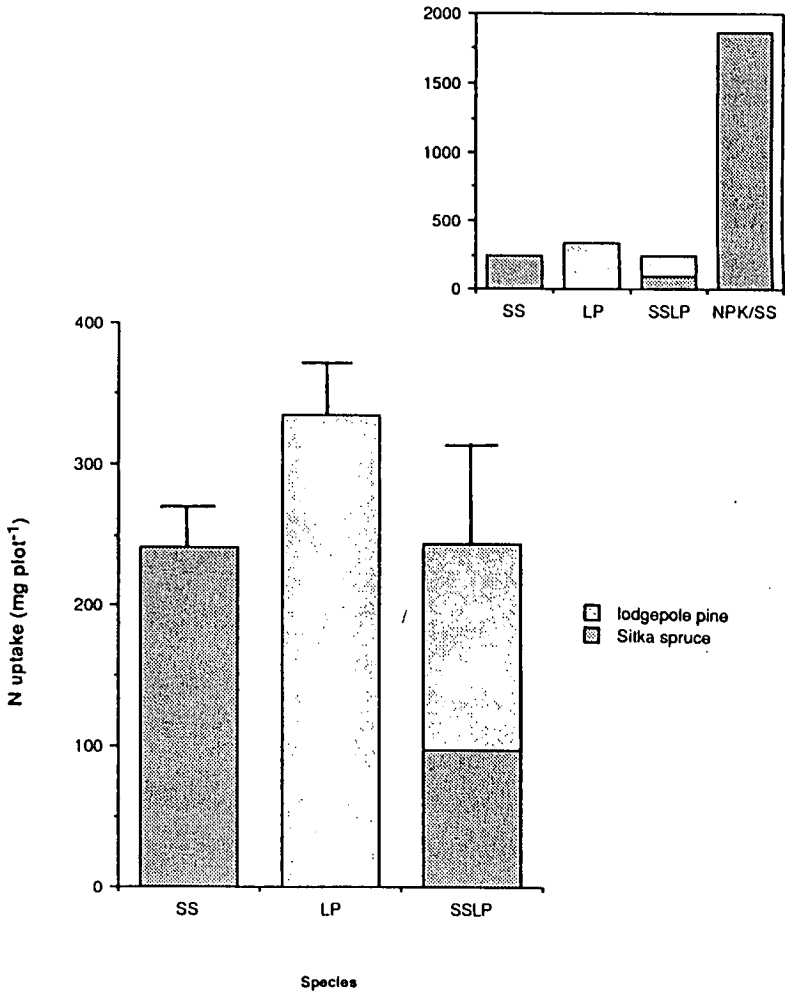


Figure 2. Mean uptake of N in PK-fertilized plots *vs* species-composition of plots. Inset shows relationship of these PK treatments to the NPK treatment. Bars show 95% confidence intervals.

P and K uptake.

In the *unfertilized* treatments, P and K uptake were considerably higher for LP than for SS (Table 4). In the *PK-fertilized* treatments the differences were smaller and less consistent.

Table 4. P and K uptake per plant (mg). ***Standard errors in italics.***
Also see Statistical Note, page 39.

Fertilization		0		PK			NPK
Species		SS	SSLP	SS	LP	SSLP	SS
P uptake	SS	$0.97_{.06}$	$0.68_{.12}$	$^{b}43.7_{0.1}$	-----	$^{ab}39.0_{5.3}$	$43.5_{1.1}$
	LP	-----	$1.18_{.11}$	-----	$^{ab}38.8_{1.5}$	$^{a}31.3_{3.0}$	-----
K uptake	SS	$4.03_{.23}$	$1.91_{.05}$	$^{a}83.2_{3.2}$	-----	$^{a}81.3_{9.0}$	$153_{5.7}$
	LP	-----	$6.93_{.30}$	-----	$^{a}94.5_{3.5}$	$^{a}73.6_{11.6}$	-----

N :P:K Quotients.

Whole-plant N:P:K quotients showed considerable variation between treatments (Table 5a), ranging from 100:3:11 (unfertilized SS) to 100:96:210 (PK-fertilized SS). With *PK fertilization*, P:N and K:N ratios were exceptionally high for both species, though they were always lower for LP than for SS. This trend was particularly highlighted in the N:P:K quotients of needles (Table 5b, columns 3-5). Here the quotient 100:51:110 for pure LP contrasts with 100:244:398 for pure SS.

Table 5. N:P:K quotients for needles and whole plants.

Fertilization		0		PK			NPK
Species		SS	SSLP	SS	LP	SSLP	SS
Whole plant	SS	100:3:11	100:3:10	100:95:182	----------	100:96:210	100:14:50
	LP	--------	100:4:15	----------	100:60:144	100:94:138	----------
Needles	SS	100:2:10	100:2:10	100:244:398	----------	100:217:385	100:13:58
	LP	---------	100:3:16	----------	100:51:110	100:53:116	----------

The high P:N and K:N ratios in PK treatments clearly highlights the problem of excessive PK fertilization in relation to mineral N supply. In contrast, in the *NPK-fertilized treatment*, where P and K were applied at the same rate as in the *PK-fertilized treatments*, the whole-plant N:P:K ratio, 100:14:50 compared reasonably well with the application rate, 100:18:66.

Changes in peat Ca and Mg concentrations.

The total nutrient concentrations in *unfertilized unplanted* controls peats, and the percentages of each nutrient extractable in 2% acetic acid (18 hrs, 20°C) are shown in *Table 6*.

Table 6. (a) total soil nutrient concentration as % of dry matter and (b) the % of total nutrient extractable in 2% CH_3COOH *(18hrs, 20°C).*

	N	P	K	Ca	Mg
(a)	1.12	0.026	0.038	0.134	0.104
(b)	<1%	14%	42%	44%	58%

In the *NPK-fertilized* tubs, *plants* depleted total Ca and Mg levels by 80% and 67% respectively. These amounts are much greater than those extracted by *acetic acid* (Table 6b), though this is hardly surprising given the frequent moisture fluctuations in the NPK treatment over the long lifespan of the experiment. Corresponding values for Ca and Mg depletion by plants in *PK-fertilized* treatments were 30 and 21% for pure SS and 58 and 33% for pure LP.

Residual free glucose and soluble carbohydrate levels.

Free glucose levels ranged between 60 μg g^{-1} (unplanted) and 730 μg g^{-1} (PK-fertilized SSLP) and soluble carbohydrate levels between 410 μg g^{-1} (unplanted) and 11,700 μg g^{-1} (PK-fertilized LP). *Mean* concentrations for each treatment are shown in *Table 7*. Notably, in the PK-fertilized treatments, mean carbohydrate levels were much higher in peats under pine than in those under spruce, and levels in the mixture peats were intermediate between the two treatments.

Table 7. Free glucose (measured enzymatically) and soluble carbohydrate (measured using anthrone) in peats de-rooted at harvesting. Units: μg g^{-1}. *Std. errors in italics.*

Fertilization	0		PK			NPK
Species	SS	SSLP	SS	LP	SSLP	SS
Free glucose	407_{35}	384_{38}	288_{23}	212_{34}	476_{86}	185_{9}
Carbohydrate	1570_{219}	2620_{411}	1290_{86}	5460_{1654}	3770_{1374}	1510_{143}

Unplanted: free glucose 60_{2}; carbohydrate 410_{10}

When the soluble carbohydrate concentrations of all PK-fertilized samples were plotted against the amounts of N taken up by plants, a good correlation was obtained ($r=0.77$; $p<0.01$)

There were no significant correlation between free glucose and soluble carbohydrate concentration. While *initial respiratory activities* did not correlate well with free glucose concentrations, there was a good correlation with carbohydrate levels ($r=0.85$; $p<0.01$). Results for the PK fertilized treatments are shown in *Figure 3*.

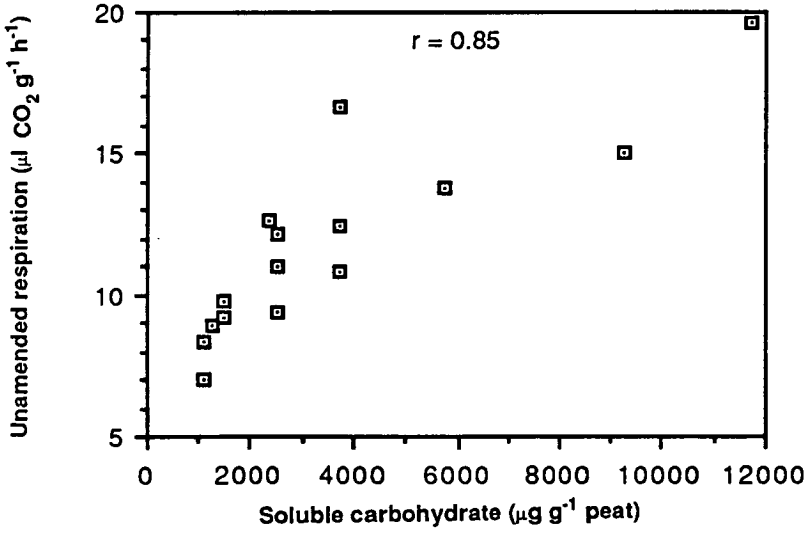


Figure 3. Unamended respiratory activities of de-rooted PK -fertilized peats *vs.* residual soluble carbohydrate levels.

An interpretative complication can be identified associated with the soluble carbohydrate results, however. Extracts from PK-fertilized treatments tended to have higher concentrations of brown-coloured material than any of the other extracts. This raises the possibility that much of the carbohydrate in these samples is not freely available, but is contained as part of humic or humic-type complexes and is only released during hydrolysis by the 20N H_2SO_4-anthrone reagent. The very low proportion of carbohydrate present as free glucose (esp. see columns 4 and 5) is unusual (*e.g.* see chapters 4 and 5) and provides circumstantial evidence to support this suggestion. The close relationship between soluble carbohydrate levels and respiratory activities, and between soluble carbohydrate levels and N uptake rates does suggest, nonetheless, that whether or not there are mechanistic relations between these variables, soluble carbohydrate levels do provide a good index of C and N release rates. This is also indicated by the close relation between carbohydrate concentrations and the mineralization rates of an amino acid amendment, reported later.

Glucose utilization over 16 hours.

In the de-rooted soil samples obtained at harvesting, the quantity of *glucose amendment* utilized over 16 hrs ranged from 470 to 2425 μg g^{-1} dw soil. This represented 19-97% of the initial amendment (2500 μg g^{-1} dw). The mean values for utilization, given as % amendment utilized, are shown in *Table 8*.

Table 8. Glucose utilized (0-16 hrs, 20°C). Values given as % amendment (viz. 2500 μg g^{-1} dw peat). ***Std errors in italics.***

Fertilization	none		PK			NPK
Species	SS	SSLP	SS	LP	SSLP	SS
% glucose used	62_2	65_1	77_2	93_2	81_2	90_1

Unplanted: unfertilized 22_2 ; PK-fertilized 28_0 ; NPK-fertilized 21_2 .

All planted treatments performed substantially better than unplanted ones and in *PK-fertilized* treatments, LP samples consistently showed higher utilization rates than SS samples (Figure 7a).

When glucose utilization values from individual samples, including those from unplanted controls were plotted against unamended respiration values, a high correlation was obtained (r=0.84; p<0.01). If the NPK treatment was excluded from the analysis (see next section) this increased to r=0.91. The results are shown graphically in *Figure 4*.

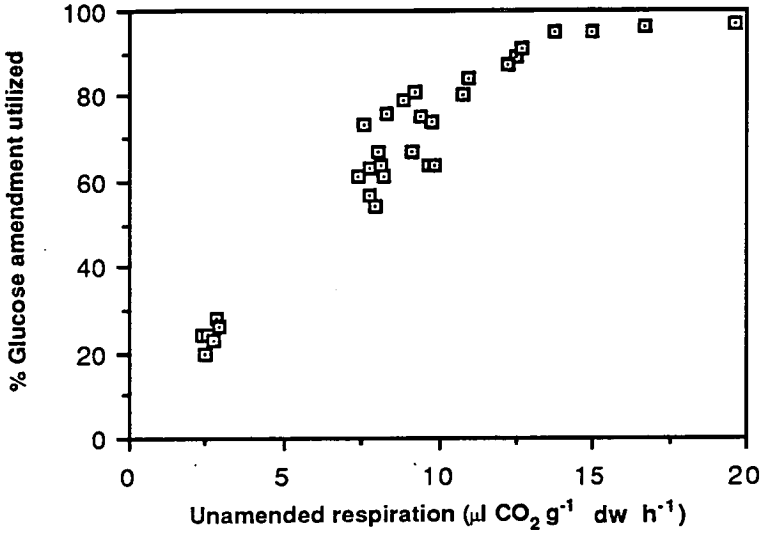


Figure 4. Utilization of a glucose amendment over 16 hrs. *vs.* unamended respiration.

Some curvature was present with the higher results suggesting that a shorter incubation period *or* a higher amendment would have been more appropriate.

Amino acid mineralization over 24 hours.

In the de-rooted soil samples obtained at harvesting, the quantity of amino acid amendment mineralized over 24 hrs ranged from 30 to 204 µg N g^{-1}. *Mean* values for each treatment are shown in *Table 9*.

Table 9. Mineral-N released (0-24 hrs, 20°C) following an amino acid cocktail amendment of 1600 µg g^{-1} amino-N g^{-1} dw peat. Units µg N g^{-1} dw. *Std errors in italics.*

Fertilization	none		PK			NPK
Species	SS	SSLP	SS	LP	SSLP	SS
N mineralized	107_{2}	101_{5}	133_{4}	169_{12}	129_{18}	217_{25}

Unplanted controls: unfertilized 40_{2} ; PK-fertilized 37_{2}

All planted peats showed much higher levels of amino-N mineralization activity than unplanted ones. For example, PK-fertilized *LP* peats released four times more NH_4 from amino acid than the corresponding unplanted peats. Also, PK-fertilized LP samples were significantly higher than corresponding SS samples (Figure 7b).

When the mineralization activities of individual samples, including those from unplanted controls, were plotted against their corresponding *unamended respiration* values (described later), a high correlation was obtained (r=0.91). In this comparison, the NPK-fertilized treatment characterised by high amino acid degradation but low basal respiration was excluded from the analysis. This omission was considered acceptable given the large *fertilizer*-N inputs to the treatment over the lifespan of the experiment and the swamping effect these may have had on root-mediated phenomena in the rhizosphere. The results are shown graphically in *Figure 5*.

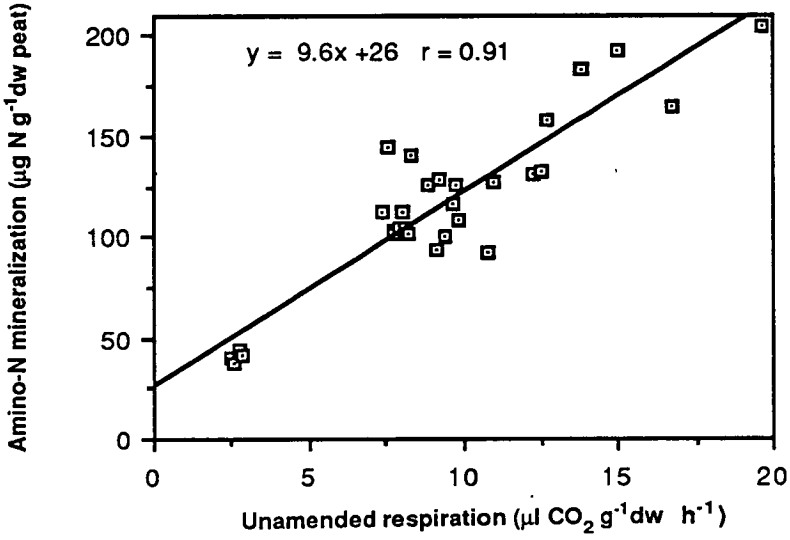


Figure 5. Mineral-N release from an amino acid cocktail amendment over 24 hrs. *vs.* unamended respiration.

When the mineralization activities of all PK-fertilized samples were plotted against corresponding soluble carbohydrate concentrations, a good relationship was also apparent (r=0.79; p<0.01).

When mineralization activities of all *PK-fertilized samples* were plotted against the corresponding *plant uptake* values, described previously, a fairly close relationship was observed (r=0.79; p<0.01). This can be seen in *Figure 6*.

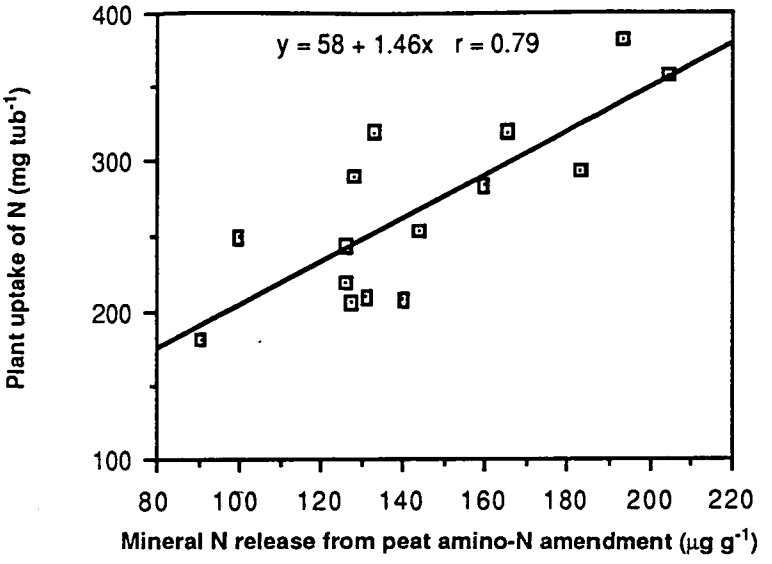


Figure 6. Amount of N taken up by PK-fertilized plants at harvesting *vs.* N mineralization activity in underlying de-rooted peats following an amino-N amendment.

C mineralization during incubation.

In the de-rooted peat samples obtained at harvesting, and subjected to a 42 day laboratory incubation, respiratory activity - as CO_2 output - was measured at intervals of up to 5 days. CO_2 output from samples over the first 48 hrs of the incubation was a very accurate indication of output over the entire incubation period (n=39; r=0.96). Mean values for treatments are shown in *Table 10*.

In PK-fertilized treatments, the presence of LP had a profound impact on peat respiration: in the first 48 hrs this was 71% higher for LP than for SS peats and 34% higher for the SSLP mixture peats than for SS ones (Table 10).

When the respiratory activities of planted PK-fertilized peats during this period were plotted against plant N uptake levels a fairly close correlation was apparent (r=0.72; $p<0.01$). This can be seen in *Figure 8*.

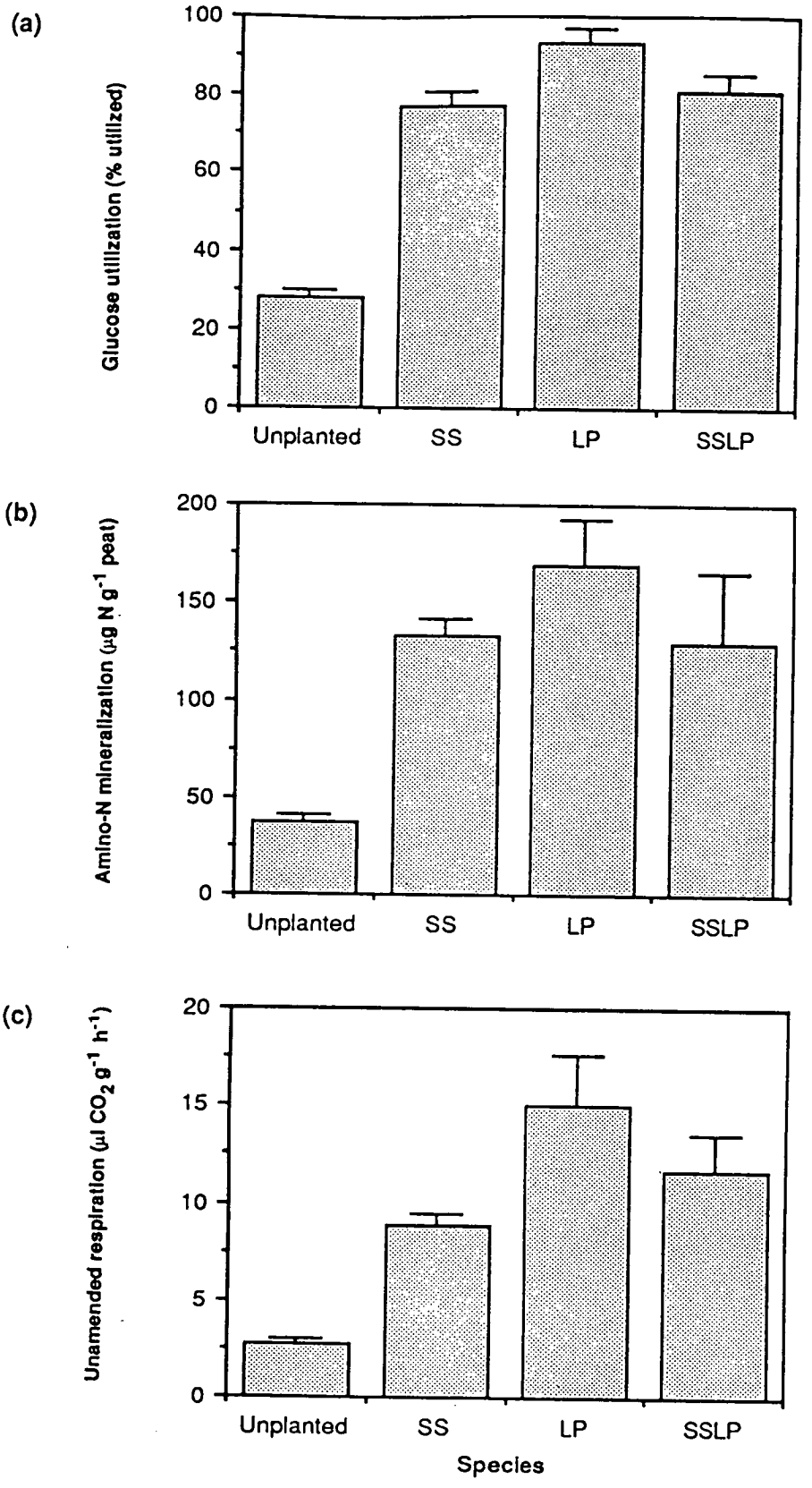


Figure 7. Charts showing (a) utilization of a glucose amendment (b) mineralization of an amino-N amendment and (c) initial respiration rate in de-rooted peats from PK-fertilized treatments. Bars show 95% confidence limits.

Table 10. Mean CO_2 output rate for each treatment over the first and last 2 days of incubation and over the entire incubation period (42 days). Units: µl CO_2 g^{-1} dw h^{-1}. *Std. errors in italics.*

Fertilization	0		PK			NPK
Species	SS	SSLP	SS	LP	SSLP	SS
days 0-2	7.84 *.15*	8.94 *.41*	8.76 *.36*	15.0 *1.3*	11.7 *0.9*	9.72 *.31*
days 40-42	3.92 *.06*	3.89 *.09*	3.60 *.14*	5.84 *.84*	5.39 *.60*	3.45 *.12*
days 0-42	4.09 *.06*	4.20 *.07*	4.06 *.18*	6.50 *.78*	5.57 *.53*	4.28 *.21*

Fertilization	0	PK	NPK
Species	0	0	0
days 0-2	2.64 *.05*	2.72 *.15*	1.95 *03*
days 40-42	2.28 *.07*	2.49 *.05*	1.57 *.04*
days 0-42	2.42 *.04*	2.64 *.03*	1.72 *03*

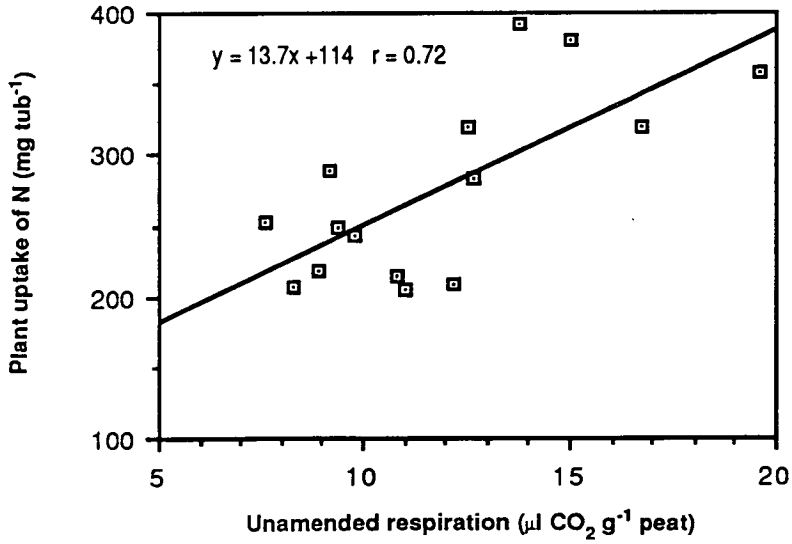


Figure 8. N uptake by PK fertilized plants during the experiment *vs.* unamended respiration of de-rooted peats at harvesting.

The differences in CO_2 release rates were greatest at the outset of the incubation and became progressively smaller with time: over the initial 48 hrs of the incubation, release rates in all planted treatments were 3.0 - 5.5 times the rates of corresponding unplanted controls while in the sixth week of the incubation, rates fell to between 1.7 and 2.3 times those of controls (Table 10). There is nonetheless, a very high correlation between a sample's CO_2 release rate at the start of the incubation and its release rate at six weeks (Figure 9). This indicates that the factors responsible for the initial variation are fairly persistent, though the intensity of their effect falls off with time. Previous research has implicated sampling disturbance as a potentially important variable for initial CO_2 release rate, however the effects have been found to be relatively short-term (<1-2 weeks). Here, in view of the high variability in initial release rates and the long-term persistence of the pattern of variation (Figure 9), it can be concluded that these results primarily reflect treatment differences rather than handling disturbance.

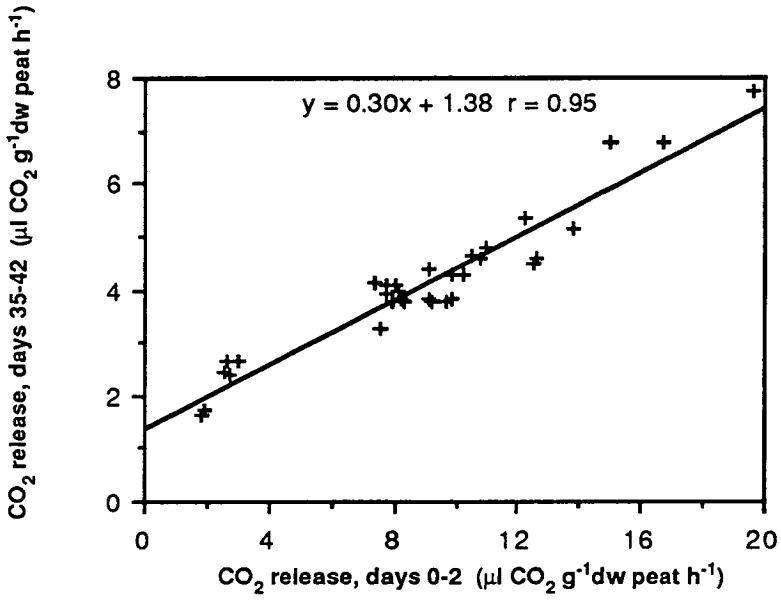


Figure 9. Comparison of CO_2 release rates from de-rooted peats at the start and end of six week laboratory incubation periods.

The potential impact that *fine root fragments remaining after de-rooting* might have on the results was quantified (see later) and found to amount to no more than 4% of measured values.

N mineralization during incubations.

At planting, residual levels of mineral N were 440 μg N g^{-1} dw. At harvesting there was virtually no mineral N remaining in any planted treatment: levels ranged between 5 and 33 μg g^{-1} and were not treatment-related. In *unplanted* treatments, levels had increased to 1050 μg g^{-1} (336mg tub) and were the same with or without PK fertilization.

N mineralization rates over the 6 week incubation period ranged from 0.33 to 5.00 μg N g^{-1} dw day. Mean values for treatments are shown in *Table 11*. Lowest rates of mineralization were associated with the *unplanted* treatments, an outcome which may relate to inhibitory levels of NH_4, rather than to the absence of plants (see discussion).

In the planted treatments, N mineralization rates during incubations were highly variable and showed no significant treatment differences. In these treatments, there was no meaningful relationship between the N levels of samples at the start of incubations and their subsequent mineralization rates.

N mineralization rates bore no relationship to *amino-N amendment* mineralization rates, to *glucose amendment* utilization rates or to basal respiration rates. As as consequence of their high variability, C:N mineralization rates were also highly variable and showed no consistent treatment differences.

Table 11. Mean N mineralization rates (μg N day^{-1}) and C:N mineralization ratios during 42-day incubations of de-rooted peats. ***Std. errors in italics.***

Fertilization	0		PK			NPK
Species	SS	SSLP	SS	LP	SSLP	SS
N mineralized day^{-1}	2.79 *.26*	2.17 *.14*	2.00 *.14*	2.43 *.76*	1.69 *.62*	2.69 *.38*
C:N mineralizn.ratio	19.4 *1.8*	25.2 *1.3*	27.1 *3.0*	31.4 *2.9*	38.5 *5.9*	22.7 *4.6*

Impact of fine root contamination on C and N mineralization.

In the samples of unplanted peat amended with pre-weighed excised fresh root tips (up to 50 mg root g^{-1} dw peat), CO_2 output attributable to roots was measured over the first 48

hrs as:

(a) 0.067 ± .004 µl CO_2 mg^{-1} root h^{-1} for SS, and

(b) 0.075 ± .006 µl CO_2 mg^{-1} root h^{-1} for LP.

The actual root weights removed under magnification from the *de-rooted* samples used in the various soil analytical procedures described here ranged between 1 and 4 mg root g^{-1} dw soil and were not treatment related. At the highest level of contamination therefore, the respiratory contribution from roots could not be expected to exceed 0.30 µl CO_2 g^{-1} dw soil h^{-1} (4 X 0.075). After 48 hrs, the respiratory activity of root tips progressively declined.

In planted treatments, basal respiration rates over the first 48 hrs ranged from 7.6 to 19.6 µl CO_2 g^{-1} dw soil h^{-1}. The maximum contribution from root tips of 0.30 µl CO_2 g^{-1} dw soil h^{-1} therefore represents a maximum contribution of less than 4% to the measured respiratory values. This indicates that respiration from soil samples is almost entirely related to decomposition activity and that no correction factor need be applied for root respiration.

Further confirmation of this was obtained from additional studies in which some rhizosphere soil samples were were separated into two portions. One of these was subjected to the usual de-rooting procedures, while the other was de-rooted less rigorously. Subsequently, sub-samples were taken for CO_2 output measurements and for root weight determination under magnification. Results indicated that atleast up to 5 mg live root tip g^{-1} dw soil could have been tolerated in the rhizosphere soil samples used in this study without any perceptible impact on measured values.

Root-amended samples were used in the studies on glucose utilization, amino acid mineralization, and N mineralization during incubation. Results indicated that the levels of root remaining in samples after de-rooting (viz. 1 - 4 mg g^{-1} dw soil) were quantitatively unimportant with respect to measured values.

DISCUSSION.

It is not clear whether N uptake by plants depends on the overall level of microbial activity. The results from PK-fertilized treatments suggest that uptake and activity at harvesting *are* closely related (r^2 = 0.67; $p < 0.01$). The comparison between fertilized and unfertilized

treatments does not support this trend, however: in the SS treatments, uptake was 28% lower with PK fertilization despite the same levels of activity. Also, in the SSLP treatments uptake is the same but activity is 68% higher with PK fertilization.

An obvious approach to such apparent discrepancies is to disregard the differences between peats removed from *different fertilizer* treatments - a step which seems reasonable, given the different array of rhizosphere conditions which can be expected under different fertilization regimes - and to compare only those differences which are present under the *same* fertilization conditions. This approach has been largely adopted in this discussion.

With PK fertilization, respiratory activity levels were on average 71% higher under LP than under SS and the difference was present in all treatment replicates (Table 10; Figure 7c). Two functional attributes of the microbial communities under LP were also consistently higher: the utilization of a glucose amendment (21% higher; $p<0.01$) and the mineralization of an amino-N amendment (27% higher; $p<0.01$).

In bulk rhizosphere peat samples, it is not possible to separate the activities of microbes associated with the utilization of labile components originating from plant roots, including mycorrhizas, from the fraction more intimately associated with the mineralization of peat components. Consequently, it cannot be assumed that differences in the decomposition rate of labile amendments reflect increased *in situ* decomposition of native peat.

If *N uptake* results, however, reflect N release rates, then it would appear that under conditions of PK fertilization *either* N release is suppressed by Sitka spruce *or* it is enhanced by lodgepole pine.

It cannot be said with certainty whether N release has been suppressed or enhanced because there is no unplanted control treatment which is strictly comparable. There are two main reasons for this. *The first* is that in these planted treatments mineral-N levels were almost entirely depleted within the first few months of the experiment (pers. data) while the corresponding unplanted controls had accumulating concentrations of NH_4: these rose to 1050 $\mu g\ N\ g^{-1}$ (336 mg tub^{-1}) over the course of the experiment. NH_4-induced repression of decomposition has been widely reported elsewhere (Keyser *et al.*, 1978; Kirk *et al.*, 1978; Fenn and Kirk, 1981; Kirk and Fenn, 1982; Berg *et al.*, 1982), though in these studies, interpretations are based on reduced C mineralization rates rather than reduced N release rates and it cannot be assumed that the two are necessarily effected in the same way

(e.g. Hendrickson, 1985).

The second main difference between the unplanted control peats and corresponding planted treatments is that the planted ones underwent larger moisture fluctuations during the course of the experiment, probably as a result of transpirational losses, and the impact of these fluctuations on N mineralization have not been assessed.

For these and other reasons, *N uptake in planted treatments* cannot be compared with *N released from corresponding unplanted peats*. As noted above, this means that it is not possible to be sure whether N release after planting has been suppressed by Sitka spruce or enhanced by lodgepole pine.

The failure of SSLP to show a mean uptake value intermediate between pure SS and pure LP could relate to the very high root density present in PK fertilized treatments and an overwhelming effect of SS roots on net N mineralization. The failure to show intermediate characteristics was also apparent in the plant biomass values (Figure 1) and in the mineralization rates of the amino-N amendments (Figure 7b). Where a positive mixture effect has been reported from the field, the ratio of spruce to pine or larch has frequently been 1:3 (Carlyle, 1984; Carlyle and Malcolm, 1986a,b) and in situations where it has been 1:1 (Miller and Miller, 1987), it is possible than the spatial exploitation of peat by SS is less intense than in this experiment.

The mechanism of apparent suppression or enhancement of peat-N mineralization remains elusive. Any speculative explanation based on these results is limited by the uncertainties surrounding the characteristics of mycorrhizal infections. Visual inspection of harvested root systems, suggested that SS roots were less heavily infected with mycorrhizas than LP roots and that within LP samples, some were more heavily infected than others.This appeared to be reflected in a higher concentration of humic material, possibly fungal melanins in acetic acid extracts of peats: in extracts of peats under spruce transmittance of light at 450 nm in 1 cm cuvettes was always 100% while in extracts of peats under pine it varied down to 93%. Production of melanins is widespread amongst fungi and has been reported for example for the ectomycorrhizal fungus *Pisolithus tinctorius* (Tan *et al.*, 1978).

Because of the uncertain implications of mycorrhizal variations, it would be unwise to assume that the species-related differences reported here are likely to exist in other situations. The possibility that some species form less effective mycorrhizal associations,

at least in the context of N nutrition, appears to warrant closer consideration however, and any future experimental studies of the type undertaken here should take account of the nature and extent of mycorrhizal infection. The very high P:N and K:N tissue concentrations present in PK-fertilized plants (Table 5) indicates that P and K applications, though considerably enhancing biomass production (Table 1), may be excessive for optimal mycorrhizal infection. This also needs to be considered in any future experimental design.

Residual NH_4 levels in the samples de-rooted at harvesting all approached zero (all <35 μg g^{-1}). Subsequent release rates during incubation were highly variable and bore no relation to treatment, to initial N levels or to sample respiration levels. It may therefore therefore be the case that release rates during incubations are not reflective of *in situ* release rates. Chemical or microbiological changes induced by disturbance during sampling - a known variable for N mineralization during incubations (This thesis, chapter 4) - and the mortality and mineralization of rhizosphere microbes, including mycorrhizas, are possible factors that could account for sample variation in N release during incubations.

Fine root contamination present after de-rooting of planted peats was found to be quantitatively insignificant with respect to measured values for residual free glucose and soluble carbohydrate concentrations, sample respiration, glucose amendment utilization, and amino-N amendment mineralization. With all these variables, results from planted treatments were considerably higher than those from unplanted treatments (see figure 7). In PK treatments, for example, residual free glucose was 3.5 times higher, initial respiration 5.5 times higher, glucose amendment utilization 3.3 times higher and amino-N mineralization 4.6 times higher in LP planted peats than in unplanted controls. After six weeks incubation, respiratory levels in peats continued to show large treatment differences which were closely correlated to initial activities (r=0.95; Figure 9). This indicates that planting leads to the development of a more favourable environment for at least some microbial activities and that these more favourable conditions persist long after roots have been removed. There is no evidence from any part of this microcosm study that these conditions favour N release processes, however.

It is not clear from these results whether the residual levels of free glucose and other soluble carbohydrates are related to root exudation or to increased extracellular enzyme activity associated with fungal hyphae permeating the native peat. Even after six weeks laboratory incubation, higher concentrations of glucose can frequently be extracted from planted than from unplanted peats (unpublished data; also see *Chapter 4)*, and this suggests

that at least some free glucose initially extracted is not in a transient state between plant roots and microbes, but is present throughout the peat and is in continuous supply as a result of extracellular enzyme activity. The formation of a reserve of soluble carbohydrate in response to extracellular enzyme activity has been recognized in decompostition studies (Swift *et al.*,1979; Swift, 1982) and has been shown to increase as well as decrease as decomposition proceeds (Frankland,1969). The addition of a *cellulase* amendment to a soil increases the level of soluble carbohydrate that can be extracted (Tateno, 1988) and this may indicate that under natural conditions there is also some delay between the release of glucose from native organic matter by free enzymes and its uptake by microorganisms.

The higher respiratory levels after six weeks incubation in those peats with high initial glucose utilization and amino-N mineralization rates support the view that the longer-term conditions for at least some microbial activities can be enhanced by the presence of roots and that some plant species and/or their mycorrhizal fungi are more favourable in this respect than others.

CHAPTER 4

(GREENHOUSE STUDY)

MEASUREMENT OF FREE GLUCOSE, RESPIRATORY ACTIVITY AND N RELEASE IN DE-ROOTED PEATS UNDER SPRUCE, PINE, LARCH AND BIRCH.

CHAPTER ABSTRACT.

Labile C is a key driving variable for microbial activities in soils. *In vitro* studies frequently show enhanced decomposition of recalcitrant substrates in response to a C supplement. A prospective consequence of labile C deficiency in oligotrophic peats is a low turnover rate of refractory N substances and a correspondly low N release rate. Vegetation releases soluble C to soils through root exudation, translocation through mycorrhizas and litter inputs. Recent studies of *de-rooted peats* under two-year-old tree seedlings have shown large differences in the levels of carbohydrate extractable in 2% acetic acid. To gain a more refined insight into the nature of these differences for different species and different seasons, a routine sampling programme was devised for replicate plots of pure and mixed cultures of greenhouse-grown, spruce, pine, larch and birch during their third year of growth. Peat moisture levels were maintained at a fairly constant 78±4% and litter was removed regularly. Free glucose concentrations were consistently higher in de-rooted peats under birch and spruce-birch plots than in any of the other treatments and showed a marked seasonal pattern, characterised by lowest concentrations in July and highest in March and December. An autumn surge in peat glucose levels was also apparent in one of two spruce-pine replicates and in some individual cores from other planted treatments. Treatment differences and seasonal variations were unrelated to the weight of live roots in plots. Mean levels of free glucose in birch plots, 333-1642 $\mu g\ g^{-1}$ dw peat, were very much higher than mean levels in unplanted controls, 86-107 $\mu g\ g^{-1}$ dw peat. Free glucose levels were closely correlated to soluble carbohydrate levels (r values > 0.97). While there was some evidence to indicate that species-related increases in peat glucose concentrations resulted in increased respiration during subsequent incubations, the effects were at best small, and appeared to be mostly over-shadowed by the impact of experimental disturbance arising from sample de-rooting, homogenization, etc. Experimental disturbance was also identified as a major impulse to mineral N release. There was no evidence of a relationship between a sample's free glucose concentration and its N release rate during laboratory incubations.

INTRODUCTION.

Recent budget analysis has indicated that on oligotrophic peats, the accumulating N capitals in mixed spruce-pine and spruce-larch plantations exceed those in adjacent pure spruce plantations (O'Carroll, 1978; Carlyle, 1984; Carlyle and Malcolm, 1986a). If differential release rates of N from peat account for differential plant uptake rates, as some results suggest (Carlyle, 1984; Carlyle and Malcolm, 1986b), then the differences in N capitals between pure and mixed plots may indicate *either* that N release is suppressed in the presence of some species *or* that it is enhanced in the presence of others.

A prospective driving variable for N release which has been contemplated is labile-C flow from plants to peats either in the form of litter or as root exudates. Previous research has indicated that the degradation of major humus constituents - lignins and humic acids - is enhanced in the presence of a labile-C amendment (Mishustin and Nikitin, 1961; Myrsha, 1966; Szegi, 1967; Ander and Eriksson, 1975; Kirk *et al.*, 1976; Kirk *et al.*, 1978; Fenn and Kirk, 1981; Kirk and Fenn, 1982). In heterogeneous field substrates, a priming effect of labile C on microbial processes is frequently reported, but interpretation is usually based on increased C mineralization (Bååth *et al.*, 1978; Foster *et al.*,1980; Sparling *et al.*, 1981; Flanagan and Van Cleve, 1983; Heng and Goh, 1984) rather than increased release of mineral nutrients. Clarholm (1985) and Harmer and Alexander (1986), however, report enhanced mineralization of substrate-N in response to C amendment.

Recent analyses of de-rooted peats under two-year-old greenhouse-grown spruce, pine, larch and birch seedlings have highlighted large differences in the concentrations of soluble carbohydrate and free glucose (J.M.Campbell, unpub.). These differences are primarily related to the identity of the overlying species rather than to other prospective variables (e.g. root density; J. Morgan pers. comm.). The amounts of free glucose may be associated with labile C exudation from plant roots; or with the depolymerization of native organic matter resulting from the enzyme activities of microorganisms. The accumulation of soluble breakdown products, perhaps representing a balance between extracellular enzyme activity and microbial uptake, has been documented for a range of substrates (Cowling, 1961; Cowling and Brown, 1969; Frankland, 1969) and may be a general phenomenon (Levy, 1982; Montgomery, 1982; Swift, 1982). Whatever the origin of the carbohydrate, the measurement of large differences in this preliminary investigation raise the possibility that more information about the presence and concentration of free glucose under

different species may lead to a better understanding of decomposition activities in the presence of plants.

To assess whether the concentration of free glucose might relate to overall microbial activity (as measured by CO_2 release) and to mineral N release during incubations, a routine sampling programme was devised for the spruce, pine, larch and birch seedlings during their third season of growth.

METHODS.

The experimental block design was constructed one year prior to the commencement of the sampling programme†. Each block, 0.75 X 0.75 X 0.18 m, had been sealed with polythene and filled to 0.13 m with peat to a dry weight density of c. 0.10 g cm^{-3}. Thirty-six 1-year-old seedlings were planted in various combinations as described below.

The peat was a poorly humified *Sphagnum* peat, pH 3.4 (1:10 peat:water mixture) obtained in a dried condition from a commercial source. Before planting, it was fertilized with a solution mixture of K_2HPO_4 and KCl at the rate of 500 μg P g^{-1}peat, and 1000 μg K g^{-1}peat (c. 140 mg P and 280 mg K per plant). Throughout the experiment, moisture levels were maintained at around 78±5%.

Treatments, comprised two replicate blocks which were chosen at random. There were seven treatments, as follows: *one* unplanted treatment; *four* pure-species treatments: Sitka spruce (*Picea sitchensis* Bong.) Carr), lodgepole pine (*Pinus contorta* Dougl), Japanese larch (*Larix leptolepis* Sieb. and Zuc.) and birch (*Betula* sp.); and *two* mixed-species treatments, spruce-pine and spruce-birch. Plants of species in mixed treatments were planted alternately. Plant spacing was equivalent to 64 plants m^{-2}

The sampling procedure was as follows: At 42 day intervals, 4 cores were taken from random points in each plot, selected using random number tables. Cores were taken using a 2.4 cm internal diameter corer and were immediately transferred to a cold-room

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† The experimental design was conceived by J. Morgan (PhD Thesis, in prep.) as part of an investigation into N uptake from peats by pure and mixed cultures of spruce, pine, larch and birch.

set at 2°C. Cores were removed on 7 occasions, between March 17 and December 16, 1986.

Sample preparation commenced within one day of sampling. Cores were broken down by hand and during careful examination any fine roots were removed with forceps. Samples of de-rooted peats were then returned to 2°C. Up to 7 days elapsed between sampling and de-rooting of samples for each set of replicates.

Laboratory analyses of de-rooted peats were carried out as follows: soluble carbohydrate, free glucose, and mineral N, P and K were extracted with 2% acetic acid (this thesis, page 19). Soluble carbohydrate was measured using an anthrone procedure (this thesis, page 21) and free glucose using a glucose oxidase-peroxidase assay (this thesis, page 23). NH_4-N was measured using an automated colorimetric method employing the salicylate-dichloroisocyanurate reaction in the presence of nitroprusside (Crooke and Simpson, 1971). The sum of NO_3-N and NO_2-N was measured by a colorimetric procedure based on a reaction with sulphanilamide and Cleve's acid after passage through a cadmium reductor (Henricksen and Selmer-Olson, 1970). P (as PO_4) was determined using an automated colorimetric procedure based on the formation of molybdate blue after reduction with ascorbic acid (Murphy and Riley, 1962). K was determined by atomic emission using a Pye Unicom Sp 9 Atomic Absorption Spectrophotometer. P and K levels were unrelated to any of the other variables measured, either within plots or between treatments, and are not reported here (see J. Morgan, in prep.). CO_2 release was measured by headspace sampling and ventilation procedures (this thesis, page 26) during six-week laboratory incubations, carried out at 84% moisture and 20°C. Concentrations of mineral N were again measured at the end of incubations and by subtraction of initial concentrations, values for N release were calculated. All mineral N was present as NH_4-N: NO_3-N was not detected.

RESULTS.

Free glucose concentrations: variation with species and seasons.

The mean free glucose concentration in unplanted peats ranged from 86 to 107 $\mu g\ g^{-1}$ dw peat over the seven sampling occasions and showed no apparent seasonal trend (Table 1). The corresponding range of mean values for *pure spruce, pine and larch*

plots was 87 to 164 μg g^{-1} dw; variation among *individual samples*, 54-370 μg g^{-1}, was much greater for these planted peats than for unplanted ones (Table 1), however *the differences* between mean values were generally not significant. Mean concentrations in *pure birch and spruce-birch mixtures* were always much higher than any of the above pure treatments ($p<0.05$) and in both cases there were marked seasonal trends, lowest concentrations occurring in June and highest in March and December (Figure 1).

Table 1. Free glucose concentrations in unplanted peat (U) and in de-rooted peats under pure and mixed cultures of spruce (S), pine (P), larch (L) and birch (B) during their 3rd growing season (March-December, 1986). S/P1 and S/P2 are two significantly different replicates.

Key: **Mean** *s.d.* range

	MAR 17	MAY 04	JUN 23	AUG 04	SEPT 16	OCT 27	DEC 16
S	153_{26}	95_{33}	98_{41}	128_{40}	120_{37}	144_{100}	164_{99}
	124-192	54-103	84-132	96-211	75-178	63-330	73-360
P	162_{56}	87_{25}	96_{25}	113_{36}	95_{46}	106_{42}	100_{34}
	109-247	59-119	70-139	79-176	64-158	56-153	61-134
L	151_{25}	87_{25}	105_{3}	141_{37}	123_{35}	104_{29}	107_{20}
	132-178	54-113	95-135	84-184	63-184	81-161	79-128
B	1642_{460}	521_{297}	333_{108}	388_{211}	504_{170}	728_{287}	1068_{269}
	1151-2167	221-973	173-462	117-737	299-933	231-1154	508-1219
S/P1	118_{20}	112_{15}	115_{13}	122_{20}	209_{80}	373_{71}	586_{101}
	91-122	97-131	90-120	95-142	161-328	285-454	463-707
S/P2	190_{43}	111_{19}	110_{15}	133_{21}	131_{21}	112_{25}	161_{38}
	153-253	97-124	88-122	129-194	114-162	92-148	122-198
S/B	487_{67}	331_{194}	293_{94}	304_{82}	340_{153}	593_{325}	701_{316}
	404-567	166-603	139-464	145-398	119-553	373-1177	262-1238
U	90_{5}	91_{25}	102_{26}	107_{23}	89_{34}	87_{16}	86_{20}
	85-96	50-119	87-116	87-148	48-147	71-108	55-128

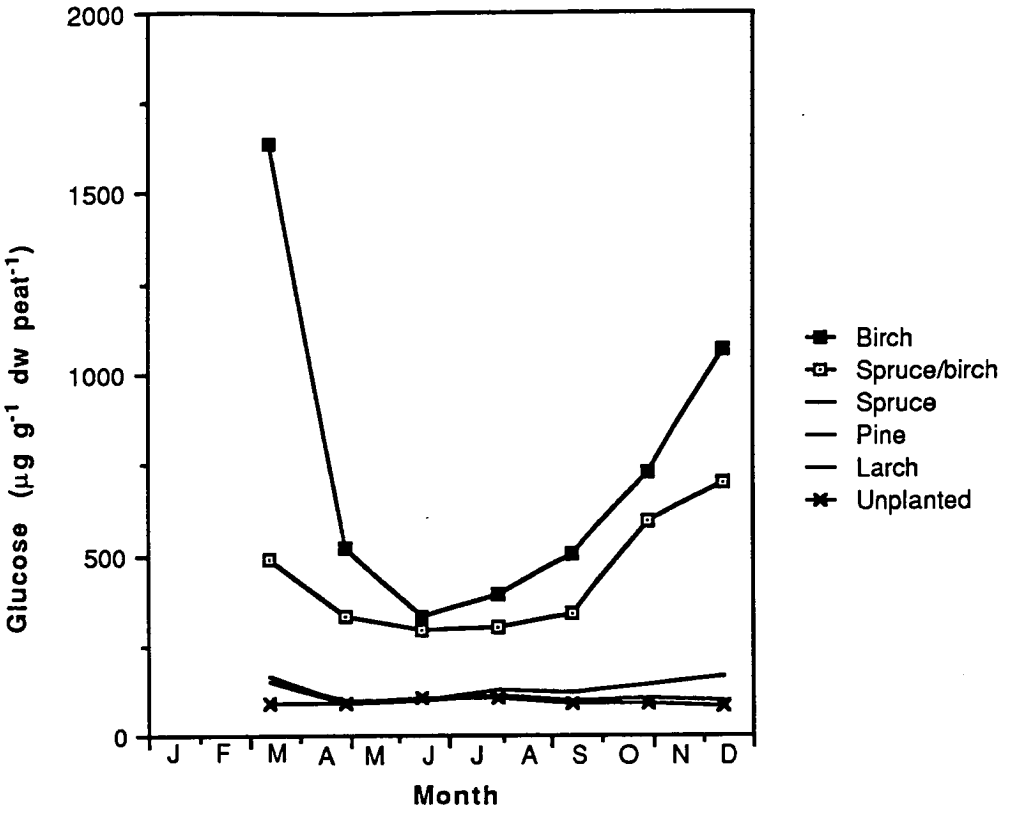


Figure 1. Mean free glucose concentrations in treatments between March and December, 1986. Spruce, pine and larch results not distinguished. Results from the two replicates of the spruce-pine treatment shown separately in Figure 2. ***Refer to Table 1 for further details.***

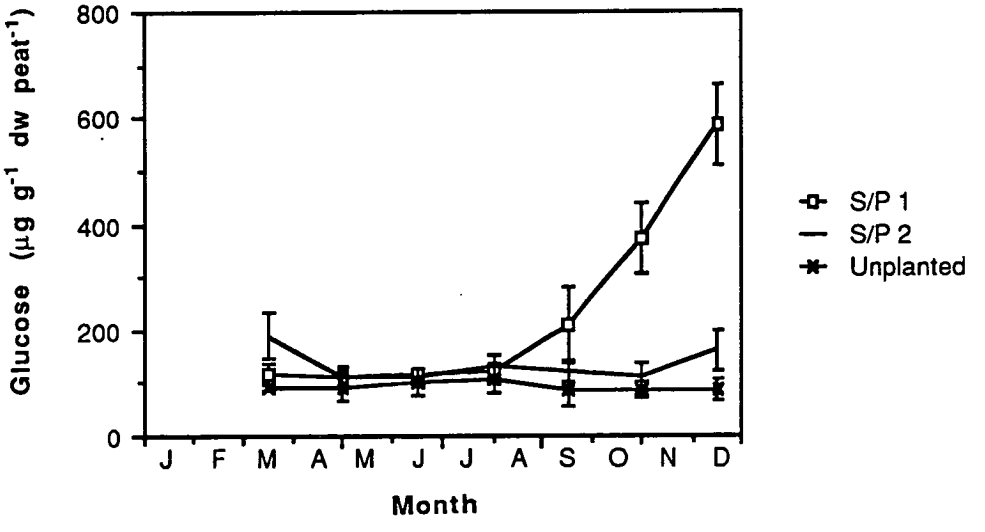


Figure 2. Free glucose concentrations in the two spruce-pine replicates, found to be significantly different, are plotted separately from the other treatments (shown in *Figure 1*).Graph also shows *unplanted* treatment concentrations for comparison.

In all the above treatments there was no significant variation between *replicate* plots and the results from both replicates could be combined to produce an overall mean and confidence interval, but in the spruce-pine treatment the two replicates showed large and significant differences. In this treatment therefore, replicates are considered individually (Table 1; Figure 2). The difference between the two replicates only emerged in early September and became increasingly pronounced through autumn and winter. This autumn surge in peat glucose levels was also apparent in the birch and spruce-birch plots, referred to above, and in some *individual samples* under pure spruce (see sample ranges, Table 1).

The mean weight of live root or the root tip number measured in peat cores (Morgan, in prep.) could not account for any of the treatment differences described: in birch and spruce-birch plots, root weight was frequently lower than in most of the other plots, yet glucose levels remained consistently higher.

While in cores within the birch and spruce-birch plots, there was often a fairly close relationship between the free glucose concentrations of de-rooted peat samples and the weight of root recovered from them, the large *seasonal* variations in these treatments could not be accounted for by seasonal changes in root growth: live root weight was higher in June than at any other time (J. Morgan, in prep.) yet glucose concentrations were at their lowest. In the two spruce-pine replicates free glucose concentration was not meaningfully related to root weight.

To reiterate, the *treatment differences* in glucose concentrations were not related to the amount of root growth. The consistently higher results in pure birch and spruce-birch mixtures suggest that treatment variability is primarily species-related. The significant differences between the two spruce-pine replicates provide an exception to these general trends, however, and indicate that additional, more subtle, variables may regulate glucose concentrations. In all cases, *seasonal* trends in glucose levels appear unrelated to changes in root growth.

Free glucose concentration as an index of soluble carbohydrate concentration.

A major drawback of the anthrone procedure for carbohydrate measurement is that has a repeatability as large as ±15–20%. This appears to relate to partial destruction of glucose during overnight drying in acetic acid. Because the variation in free glucose concentrations of the samples used in this study was considerable on each sampling

occasion, however (up to 25 fold - e.g. 85 to 2167 μg g^{-1} - see *March sample ranges*, Table 1), the anthrone procedure still offered the prospect of providing soluble carbohydrate values which could reasonably be compared to corresponding free glucose concentrations. In March and May the correlation coefficients between the two variables were 0.99 and 0.97 respectively.

In view of this close relationship, it was assumed that the free glucose fraction could provide a close reflection of soluble carbohydrate concentrations. The relationship was investigated again at the end of the sampling period for one set of replicates and a high correlation was obtained (Figure 3). The regression line on this figure, $y = 1.18x + 354$, demonstrates a general trend: increased soluble carbohydrate levels can be accounted for almost entirely by free glucose.

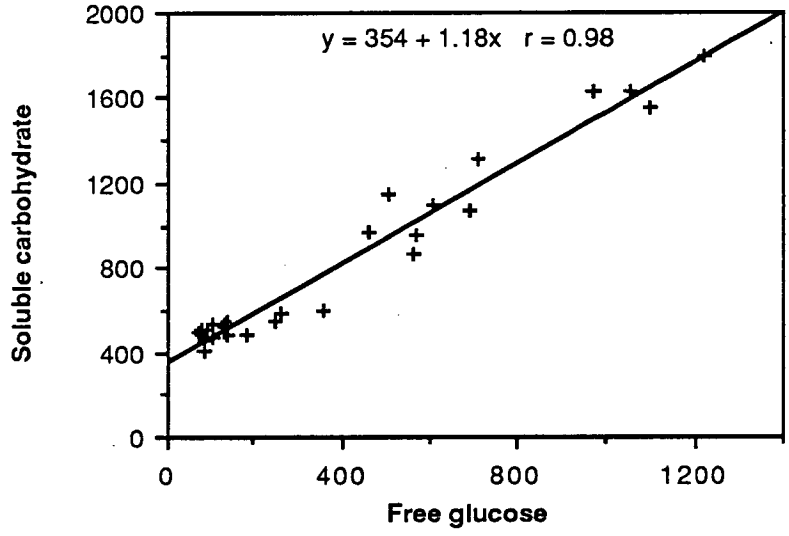


Figure 3. Free glucose (measured enzymatically) *vs.* soluble carbohydrate (measured using anthrone). Units: μg g^{-1} dw peat. (Dec 16 data).

Free glucose concentrations at the end of incubations.

Free glucose concentrations always declined over 42 day incubation periods and by the end of incubations, concentrations in most samples fell into the small concentration range, 50-200 μg g^{-1}. There were some exceptions, however, where post-incubation levels went as high as 350 μg g^{-1}. The samples with the highest glucose concentrations at the end of incubations were generally those with the highest initial concentrations. Even in unplanted controls, where concentrations approached the limits of accurate measurement -*viz.* c. 0.5 ppm (50 μg g^{-1} peat) - it was clear that there was a consistent trend towards reduced concentrations after incubations, levels falling by around 30-50 μg g^{-1}.

CO_2 release during incubations.

CO_2 release rates of samples were always highest in the first week of an incubation and became progressively lower with time. Some examples of this are shown in *Figure 4*. On most sampling occasions, the variation *within plots* was relatively high with respect to the variation *between treatments* and perhaps as a result of the small sample sizes (*viz.* n=4 individual cores), no significant differences emerged which could be related to treatments.

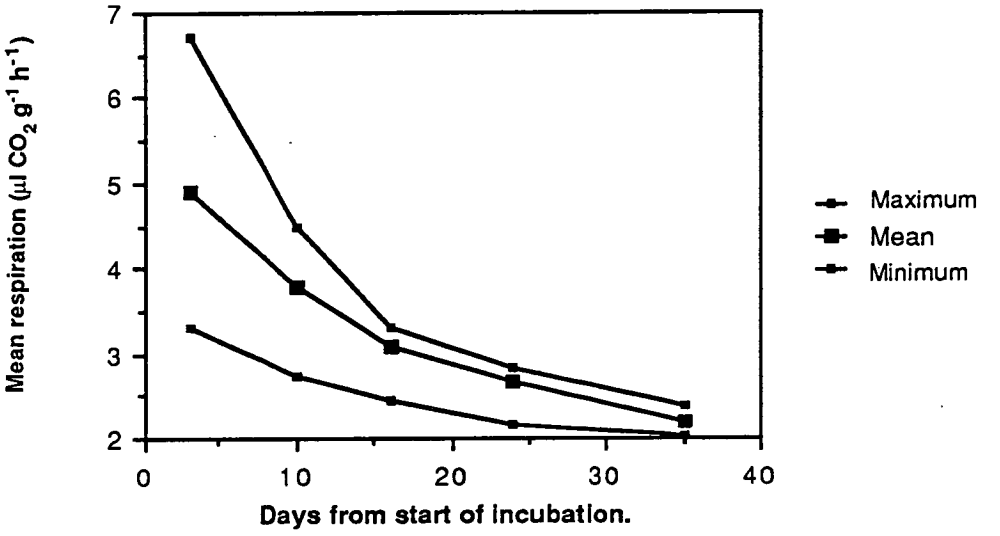


Figure 4. Example of CO_2 release profiles during incubations (Aug 04 data).

An average CO_2 release rate for each treatment over *all* sampling occasions was calculated (*i.e.mean of 7 sequential treatment means*) in order to assess if treatment differences might emerge which were not apparent on *separate* sampling occasions. When this was done, the birch treatment was found to have the highest CO_2 release rates (Figure 5). The spruce-birch and spruce-pine treatments also had high CO2 release rates but no higher than the unplanted treatment, and overall, there was no significant relation between treatment CO_2 release rates and corresponding mean levels of free glucose.

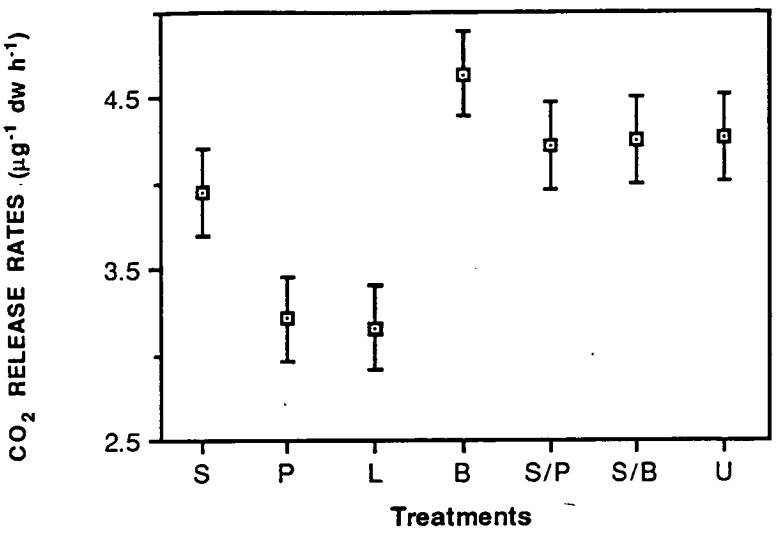


Figure 5. Mean CO_2 release rates of treatments during incubations. Each value based on 7 treatment means obtained at 6-weekly intervals between March 17 and December 16. 95% confidence limits calculated using the *T' Method*. Abbreviations for treatments as in Table 1.

The *relationship* between sample CO_2 release rate in the first week of an incubation and release in the second or third weeks were generally close with r values usually exceeding 0.80 (significant at $p<0.01$). The relationship always deteriorated with each successive week, however, as shown in *Table 2*, indicating that *the factors responsible for the initial variation are short-term in their effect.*

Table 2. Correlations between CO_2 release in the first week and release in subsequent weeks during a 6-week incubation of one set of replicates (n=28). The deteriorating relationship shows the short-term nature of the factors responsible for the initial variation (Aug 07 data).

week	2	3	4	5	6
r value	0.84	0.79	0.66	---	0.55

N release during incubations.

In the *unplanted* treatment, mean mineral N levels over the sampling occasions ranged between 121 and 142 $\mu g\ g^{-1}$. In *planted* treatments levels ranged between 9 and 47 $\mu g\ N\ g^{-1}$ and showed no apparent treatment-related trend. Subsequent N release rates during laboratory incubations showed high variability within plots and no marked or

consistent differences emerged between *planted* treatments. Release rates in all these treatments, however, were always lower than those in *unplanted controls* (Table 3):

Table 3. Table showing the relationship between residual levels of mineral-N at sampling and subsequent N release rates during laboratory incubations. (Combined results from all sampling events).

	Unplanted	Planted
Residual mineral N ($\mu g\ g^{-1}$)$_{s.d.}$	$\mathbf{134}_{13}$	$\mathbf{28}_{9}$
Range	121-142	9-47
Mean release rate ($\mu g\ g^{-1}week^{-1}$)$_{s.d.}$	$\mathbf{22.4}_{3.0}$	$\mathbf{10.0}_{2.3}$
Range	19-27	6-18

N release rates of samples during incubations either *within unplanted plots* or *within planted plots* bore no relation to initial N levels, to CO_2 release rates or to initial glucose levels. Additional research, described below, implicates handling disturbance arising from sample homogenization as a major perturbation which increases CO_2 release in subsequent weeks and which has an even more marked impact on N release.

Impact of initial disturbance on the release of CO_2 and mineral N during incubations.

The absence of a relationship between sample glucose concentration and respiratory activity, frequently observed in other studies (e.g. this thesis, chapter 5) raised the prospect that disturbance induced by handling (de-rooting/mixing/homogenization) was a potential perturbation that might effect CO_2 release and possibly also mineral N release.

Supportive evidence for this emerged from a study of the two unplanted plots: between March and December no *in situ* increase in mineral N concentrations between sequential sampling could be detected:

Mar	May	Jun	Aug	Sept	Oct	Dec	
130	139	132	121	124	124	142	$\mu g\ N\ g^{-1}$ peat.

while sequential laboratory incubations over this period consistently showed high rates

of release (Table 3), with an overall mean release rate of 22.4 μg g^{-1} dw $week^{-1}$ (Table 3). Over the sampling period (*viz.* 6 X 6 weeks) this represents 850 μg g^{-1} dw. Even allowing for the lower temperatures under greenhouse conditions *in situ* release rates between March and December ought to have shown significant incremental increases between sampling events. Given that there was no net increase, these results suggested that N release during incubations was an artefact related to experimental manipulation rather than an index of *in situ* release.

To investigate this possibility further, a complete unplanted plot of peat (0.75 X 0.75 X 0.13 m) was transferred to a constant temperature chamber maintained at 20 ± 0.5°C and left for one month to equilibrate. Moisture levels were maintained at 80 ± 2°C. Six 6cm int. dia. plastic tubes were then fully inserted into the block; three of these were removed at random and the peat inside each was thoroughly mixed by hand and then repacked to its previous density. These tubes were then returned to their original core holes in the peat block and left overnight. The following day, 10 ml graduated glass vials containing 2N KOH were placed on the surface of the peat inside each of the six plastic tubes and the tubes were then fitted with air-tight plastic caps. After one week, the glass vials were removed and CO_2 trapped in KOH was measured using the combined acidification-headspace sampling procedures described previously (This thesis, page 28).

In this first week, 27% more CO_2 was released from disturbed cores than from undisturbed ones (4.8 vs 3.8 ml CO_2 g^{-1}dw h^{-1}; significant at $p<0.05$). By the third week, the difference was no longer significant. Over six weeks there was no net change in *mineral N* levels in the undisturbed cores, however in the disturbed cores, levels increased at a mean rate of 11.9 μg N g^{-1} dw $week^{-1}$.

These results show that handling disturbance prior to incubation is a key perturbation with respect to subsequent CO_2 release from peats and that it has an even more profound effect on mineral N release.

It is clear from these results that mineral N released following the incubation of homogenized peats bears no relationship to *in situ* release rates. Although it cannot be assumed that the results are applicable to acid peats in general, the outcome appears to cast serious doubt on the uncritical use of incubations in peat-N mineralization studies.

DISCUSSION.

During the course of these studies, three types of effect have been identified in peat samples:

(1) *Plant induced* increases in the concentration of free glucose and other soluble carbohydrates.
(2) *Plant induced* reductions in the residual concentrations of mineral N.
(3) *Experimentally induced* increases in C and mineral N release rates through disturbance (homogenization, subsampling *etc.*).

The main aim of this research program was to assess the effect of plant induced increases of free glucose on CO_2 and mineral N release during incubations. It is clear however, that this is only possible if the significance of the other two impacts - (2) and (3) - can be evaluated.

Plant induced increases in free glucose concentration at the levels observed here for *birch* would probably have been reflected in CO_2 release rates during incubations if they had been the main source of variation. A concentration of 1000 $\mu g\ g^{-1}$ for example would yield 0.75 ml CO_2 on complete degradation. Even with a microbial conversion efficiency as low as 0.50 this would still have corresponded to a release rate of 2.6 $\mu l\ CO_2\ h^{-1}$ over the first week. The *variation* in release rates from samples was usually about 3.0 $\mu l\ CO_2\ g^{-1}\ h^{-1}$ in the first week of all incubations (e.g., 3.3 - 6.7 $\mu l\ CO_2\ g^{-1}\ h^{-1}$, see *Fig.4)* and given that this variation generally showed no significant relation to treatment, it has to be assumed that the potential relation between glucose and CO_2 release has been overshadowed by other factors (e.g. disturbance). When results were accumulated over the whole sampling period to assess if glucose measured in the birch treatment had an overall effect on CO_2 release which was not apparent at each sampling occasion, it was found that release from birch peats was indeed higher than from many of the other treatments (Figure 5), but overall, the differences were not consistent.

The significance of *plant induced reductions in residual mineral N levels* on N release rates during subsequent incubations cannot be readily assessed. Residual mineral N levels were always higher in unplanted treatments than in planted ones, and subsequent N release rates were also always higher. *Within* planted treatments or *within* unplanted ones, however, there was no relation between initial N level and subsequent release rate. A prospective explanation for these results is that levels of

mineral N in planted treatments are sufficiently low to inhibit microbial N release activities but that *within* planted treatments or *within* unplanted treatments the effect of smaller-scale variations in initial N (Table 3) are overshadowed by other factors (e.g. disturbance).

Further evidence that low residual N levels may be limiting for microbes emerges from the CO_2 release results. These show that mean CO_2 release in the *unplanted* treatment was higher during incubations than in some *planted* treatments (Figure 5).

Experimentally induced increases in C and N release rates as a result of disturbance was demonstrated by the additional experiments reported here on a block of unplanted peat. These showed that C release was increased by 27% following disturbance and that N release did not occur unless samples were disturbed. This indicates that disturbance may have had an overwhelming effect on C and N release from samples from planted peats. It is not possible on the basis of these results, however, to indicate precisely what aspect of disturbance provides the impulse to C and N release.

It has been reported elsewhere that disturbance has a profound impact on N release (Runge, 1974; Nordmeyer and Richter, 1985; Raison *et al.*, 1987). Raison *et al.* (1987), for example, found that disturbance, associated with sieving and mixing, increased N release during incubations by between 2 and 10 times.

From the results presented here, it has to be concluded that N uptake by plants must remain the best measure of the N supplying capacity of peats. In the previous chapter it was shown that N uptake by plants in PK-fertilized peats was closely correlated to CO_2 release from de-rooted peats; as well as to the rate of mineral-N release from an organic-N amendment. It was also shown that the effects of disturbance during sampling were overshadowed by the effects of plants. It is not clear why in this experiment the reverse appears to have been the case. Plant N uptake results have still to be reported (Morgan, J. in prep.). In the previous study, the peats were more highly humified and unplanted treatments showed substantial *in situ* increases in mineral N concentrations, increasing from 440 $\mu g\ N\ g^{-1}$ at the rate of 6.9 $\mu g\ N\ g^{-1}\ week^{-1}$. In this study, peats were poorly humified and *in situ* N concentrations in unplanted treatments showed no net increase. It is not clear, however, whether these explicit differences might be used in an explanation of the relative effects of plants and disturbance.

It has to be said that no definite information has emerged from this investigation on the

origin of the free glucose in planted peats. Nor is it clear whether the glucose measured days or weeks after a sample has been incubated is part of the same pool of glucose measured initially. Glucose in peats may be mainly associated *either* with a soluble C pool resulting from root exudation, or with a less accessible pool resulting from the activities of extracellular enzymes which have diffused beyond the immediate influence of their host microorganisms. The cellulolytic activities of *free* enzymes have been demonstrated by microscopy (Bravery, 1971) and their role in the formation of a soluble C reserve has been widely recognized (Cowling, 1961; Cowling and Brown, 1969; Levy, 1982; Montgomery, 1982; Swift, 1982).

To conclude, this study has shown that large amounts of free glucose can be extracted from de-rooted peats, and that the amounts extracted vary with different species and different seasons. There is no *clear* evidence that these amounts have a significant influence on CO_2 release during subsequent incubations, however, and no evidence *at all* that they influence subsequent N release rates. It has to be remembered that the process of de-rooting a sample may quickly bring to an end the influence of plant roots on the soil microflora and that the results obtained in incubations, therefore, do not necessarily reflect what goes on in the presence of plants.

CHAPTER 5.

(FIELD STUDY)

ROUTINE MEASUREMENT OF FREE GLUCOSE, RESPIRATORY ACTIVITY AND N RELEASE IN DECOMPOSING FIELD SUBSTRATES.

CHAPTER ABSTRACT.

In acid organic residues, it is not yet clear what substrate variables most influence trends in net N mineralization. It appears, however, that phenolic humus constituents are major sinks for the N which passes through microbial biomass as decomposition proceeds and the turnover rate of these may therefore be a major determinant of the release rate of N. Soluble carbohydrate is a major regulatory factor in microbial population dynamics and has been identified as a propellant for the *in vitro* microbial decomposition of two main groups of phenolic humus constituents - lignins and humic acids - but the field implications of soluble carbohydrate availability on mineral-N dynamics are unclear. If differential uptake of N with different species or environmental regimes could be associated with labile-C availability in the forest floor, it would raise the possibility of labile-C being a driving variable for N mineralization. Substantial quantities of soluble carbohydrate, comprised in large part of free glucose can be readily measured in extracts of forest floor materials including well-humified FH material and diverse underlying peats. Under specified conditions the quantity of glucose extracted from a sample generally provides a good indication of its respiratory activity. Glucose can be extracted from a substrate even after it has been ground and incubated for many weeks. A prospective explanation for the presence of free glucose is the presence and activity of extracellular enzymes which have diffused beyond the immediate influence of their source microorganisms. The amount of free glucose extracted may represent a balance between the supply of glucose by extracellular enzymes and its uptake by microorganisms. The measurement of glucose in laboratory *perturbation* studies should indicate whether its more widespread application to field studies will contribute towards a more refined understanding of decomposition in recalcitrant substrates.

INTRODUCTION.

Most forested peatlands in upland Britain and Ireland receive inputs of fertilizer P and K, but not fertilizer N (McIntosh, 1981,1983). On these sites, the N released during the decomposition of native detritus is likely to be the main source of N for plant uptake, and it is a good generalization that N release rates are limiting plant growth rates.

While the apparent constraints for microbial processes in upland peats - substrate recalcitrance, low pH, low Eh, toxic inhibition, low energy and mineral nutrient availability and low temperatures - may collectively appear formidable, no clear insight into the relative importance of each factor has yet emerged (Dickinson,1974). This is probably because it is barely possible to manipulate one variable without affecting others (Heal, 1981) and consequently to disentangle the *effects* of one variable from those of another.

In the past, the impact of *different forest communities* on N mineralization has been assessed only at a gross comparative level: overall growth performance, forest N uptake, microbial biomass, enzyme activities, N mineralization rates during incubations etc.(Fisher and Stone, 1969; Jones and Richards, 1977,1978; O'Carroll, 1978; Parkinson *et al.*, 1980; Nadelhoffer *et al.*, 1984; Carlyle, 1984; Turner and Franz, 1985; Carlyle and Malcolm, 1986a; Fisher and Gosz, 1986). Meanwhile, prospective driving variables for N mineralization have been obscured by numerous *plant variables* - moisture uptake; quality and quantity of litter inputs; root density; mycorrhizal associations; root exudation etc. - acting in different ways on the native *substrate variables* which constrain microbial processes (noted above) as well as introducing others (Gadgil and Gadgil, 1971, 1975).

Another more fundamental problem in identifying the driving variables for N release under forest communities is that even with more closely controlled laboratory-based research, large gaps still exist in the understanding of which substrate variables best account for release rates. The consequence for field studies is that no clear direction has emerged of *what to observe.* Previously, emphasis was frequently placed on the concept of *a fixed critical C:N ratio* (the C:N ratio at which a substrate starts to release its N) *which supposedly approximated to the ratio in decomposer biomass* (Mulder *et al.*, 1969; Alexander, 1977; Packham and Harding, 1982) but in the light of considerable experimental (e.g. Meentemeyr, 1978; Åber and Melillo, 1980; Berg and

Staaf, 1980; Berg *et al.*, 1982; Staaf and Berg, 1982b; Flanagan and Van Cleve,1983; McClaugherty and Berg, 1987) and theoretical (*e.g.* Bosatta and Staaf, 1982; Bosatta and Berendse, 1984; Bosatta and Ågren, 1985) research, this concept can no longer be upheld: it is clear that a critical C:N ratio can very considerably from one substrate to another and that they depend on characteristics which are not yet well understood.

Recent attention has focussed more on the chemical nature of substrate-C and -N constituents and particularly on the retentive power of lignin or its degradation products for substrate-N (Berg and Staaf, 1981; McClaugherty and Berg, 1987). According to Berg(1986), it appears that *the factors which influence lignin decomposition rates in turn influence N release rates. In vitro* studies have demonstrated the importance of soluble carbohydrate in lignin decomposition (Ander and Eriksson, 1975; Hiroi and Eriksson, 1975; Kirk *et al.*, 1976; Kirk *et al.*,1978; Kirk and Fenn, 1982) while enhanced mineralization of substrate-N in response to labile-C amendment has also been reported from laboratory incubation (Harmer and Alexander, 1986) and microcosm (Clarholm, 1985) research. In field studies, enhanced N mineralization is sometimes attributed to the presence of labile-C (Weber *et al.*, 1985), but in most cases only enhanced C mineralization is reported (~~Connors *et al.*, 1976~~; Bååth *et al.*, 1978; Foster *et al.*,1980; Sparling *et al.*, 1981; Flanagan and Van Cleve, 1983; Heng and Goh, 1984) and the implications for the mineralization of native substrate-N have not been examined. In the absence of more extensive research therefore, the general implications of labile-C availability for the decomposition of field substrates remain uncertain.

As part of an overall aim of moving towards a comprehensive and integrated experimental approach to decomposition in diverse substrates, recent work has established that substantial quantities of labile carbon can be extracted from decomposing substrates (this thesis, chapter 2). When applied to de-rooted peat samples under a variety of species and species-mixtures (n=64) up to an 18-fold difference in mean free glucose concentrations was apparent (this thesis, chapter 4). As reported, these differences were predominantly related to the identity of the species, rather than to other prospective factors (e.g. root mass; Morgan, pers. comm). As an extension of this work on *litter-free* soil, a research project was initiated to look at labile carbon concentrations under *litter-rich* conditions. The immediate objective of this research was to test some rapid and simple procedures for the measurement of labile carbon and respiratory activity, while a longer-term objective was to develop a

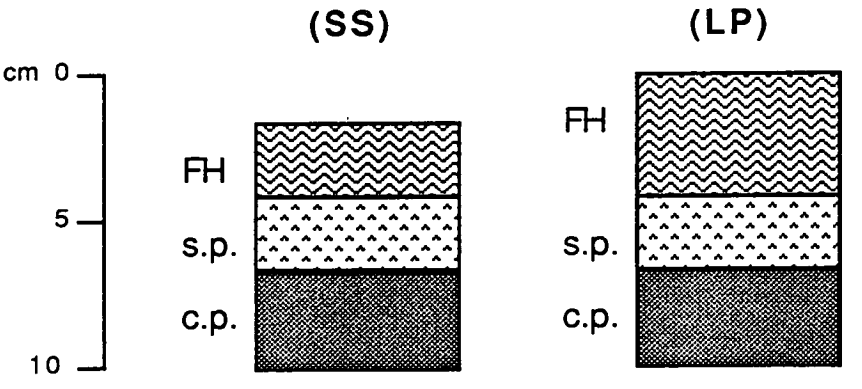
routine experimental basis for investigating the role of labile-C in mineral-N dynamics. Material underlying two species - Sitka spruce (here abbreviated to SS) and lodgepole pine (here abbreviated to LP) - was examined in the investigation.

METHODS.

The field site was located on an upland raised bog at Leadburn, some 18 km south of Edinburgh at an elevation of 280 m. The area has a mean annual precipitation of c. 1000 mm. Most of the bog was drained and planted with Sitka spruce (*Picea sitchensis* (Bong.) Carr) and lodgepole pine (*Pinus contorta* Dougl) in 1967. On the south eastern edge, a species trial was also established in 1967. Site preparation was carried out using a single mould-board plough at a spacing of 1.8 m and to a depth of 0.60 m. Plots comprised 8 rows of 12 trees. Tree spacing was equivalent to 3700 trees ha^{-1}. P and K were applied to the plots at planting and subsequently at 4-6 yr intervals at the rate of c. 80 kg P ha^{-1} and 100 kg K ha^{-1}. An area of replicates has since been clear-felled, leaving just one plot per species. Field sampling was carried out in the Sitka spruce (here abbreviated to SS) and lodgepole pine (here abbreviated to LP) plots as described below.

The field sampling procedure was as follows: 7 cores were taken at 35 - 40 day intervals from randomly selected points on the flat ploughing position in SS and LP plots. The perimeter buffer rows in plots were not sampled. Cores were taken using a 6.5 cm internal diameter corer. Any fresh litter on the surface of cores was removed and treated separately. Cores were returned to the laboratory and stored at 2°C.

Sample prepararation commenced within 1 - 2 days. Each core was removed in turn and split into three distinct layers: an FH horizon; an underlying poorly-humified sphagnum peat layer (s.p), a legacy of the pre-plantation surface vegetation; and a basal dark brown amorphous calluna peat (c.p.) :



Each of the three layers, henceforward referred to as a *substrate* or *substrate-type* ,was broken down by hand and during careful examination any fine roots were removed with forceps. The samples of each substrate were returned to 2°C until these *de-rooting* procedures were completed, some 5-7 days after sampling. On the first sampling occasion, the samples from each of the six substrates (2 plots x 3 layers) were bulked and five replicate samples were used in subsequent analyses. This was done with a view to assessing replicate variation and to highlighting potential procedural complications. Thereafter samples were analysed separately (n=6X7=42). Each core was identified by number so that results obtained from its 3 substrate-types could be compared. Samples were collected on 7 occasions between March 17 and December 04, 1986.

Laboratory analyses were carried out as described previously (chapter 2). To summarize, moisture levels in samples were determined after over-night drying at 85°C. One set of 1.5 g sub-samples was weighed into 250 ml flasks and made up to 150 ml with 2% acetic acid. Material was extracted over-night (18 hrs) on a rotary shaker and then filtered through Whatman No. 42 filter paper. Extracts were stored at 2°C and analysed for NH_4-N, NO_3-N, P and K as described previously (this thesis, page 62). A further set of sub-samples with the fresh-weight (fw) equivalent of 1.5 g dry weight (dw) was weighed into 250 ml winchester bottles and moisture levels brought up to 84% (as % fw) using a fine mist spray and a plastic stirrer. Bottles were *loosely* capped and left to equilibrate at 20° ± 0.5°C for 24 hrs. They were then fitted with two 25 X 3mm silicon rubber discs (Esco rubber, Sterilin, UK) held firmly in place by plastic 28mm R3 open screw-caps. Head-space CO_2 concentrations were measured after 5 days using a *Leybold-Heraeus Binos* CO_2 analyser and ancillary apparatus as described previously (this thesis, page 26). Open screw-caps and seals were then removed for c. 2 hrs, and bottles were loosely capped to reduce moisture loss and left over-night at 20°C. The following day bottles were again sealed using silicon discs as described above. Subseqent sampling and ventilation periods were carried out at 4 - 7 day intervals. Care was taken to limit maximum headspace CO_2 concentrations to c. 1.5%. After a total of 60 days incubation, samples were extracted in 2% acetic acid as described above.

RESULTS.

Respiratory activity during incubations; glucose levels: temporal variations; glucose levels: spatial variations; glucose concentration *vs.* initial respiration; glucose concentration at the end of incubations; glucose as an index of labile carbon concentration; accessibility of native labile carbon; contribution of fresh litter and fine roots to respiratory activity and glucose concentrations; N mineralization and C:N mineralization ratios during incubations.

Respiratory activity during incubations.

The respiratory activity of a sample over the first few days of an incubation was generally a very good index of activity over entire 60 day incubation periods. When the two variables were plotted against one another for the two bivariate-normal groups that made up each data set (FH substrates, n=14; and both peat substrates, n=28), r values were usually above 0.90 and in all cases exceeded 0.84. Values for July and August, shown together in *Figure 1* typify the form of plot obtained for each sampling occasion. Respiration over the final few days of incubations (days 55-60) also correlated well with initial respiration levels with r values usually above 0.86 and in all cases above 0.82.

Respiratory profiles during incubations followed the same general trends as the examples shown in *Figure 2*. The steep initial decline was common to all substrates, and the relative differences which this shows between litter, FH and peat substrates were very consistent and are reflected in the C mineralization values, described later.

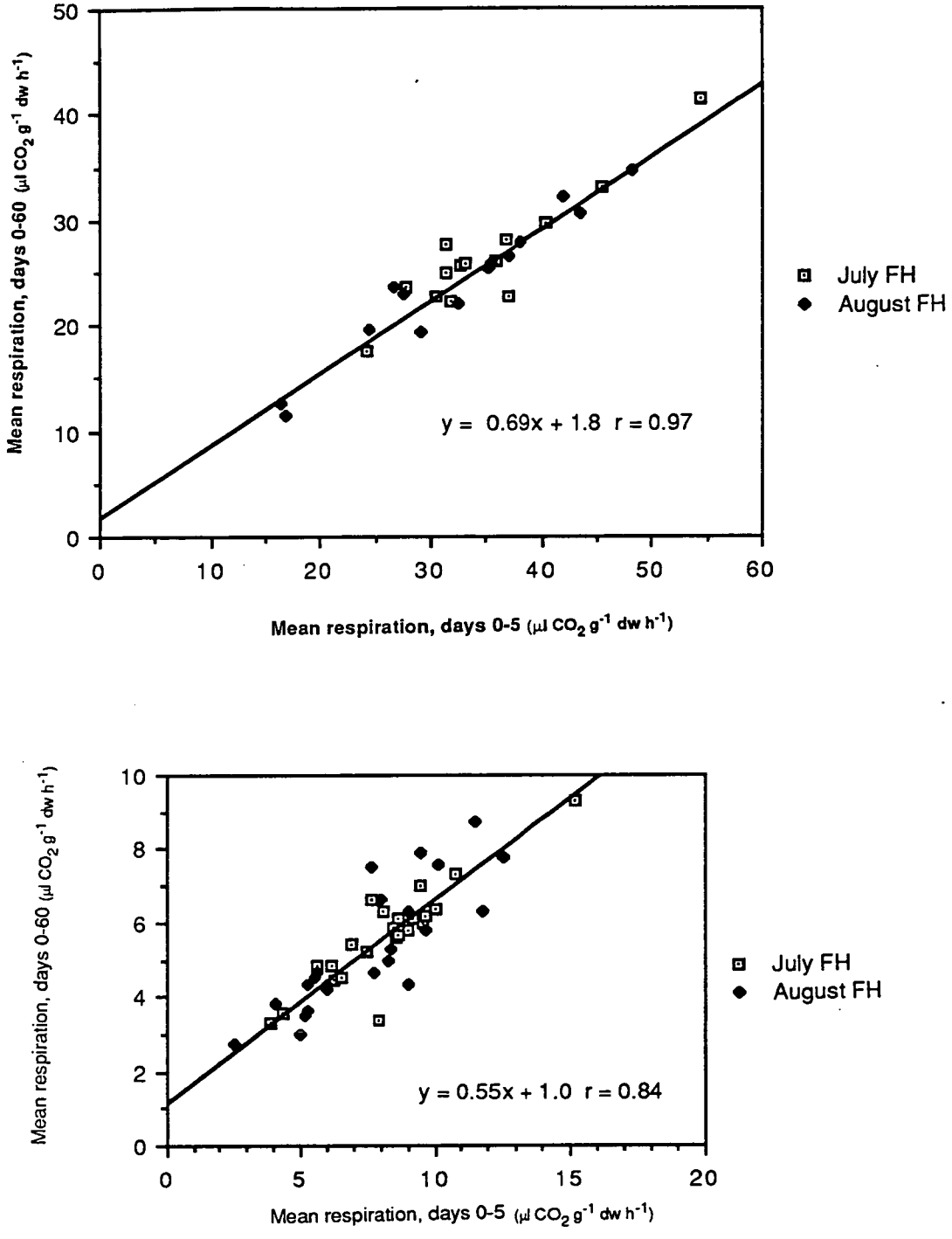


Figure 1. Initial respiration (0-5 days) *vs.* respiration over entire incubation period (0-60 days) in (a) FH material and (b) underlying peats. Combined results from July and August.

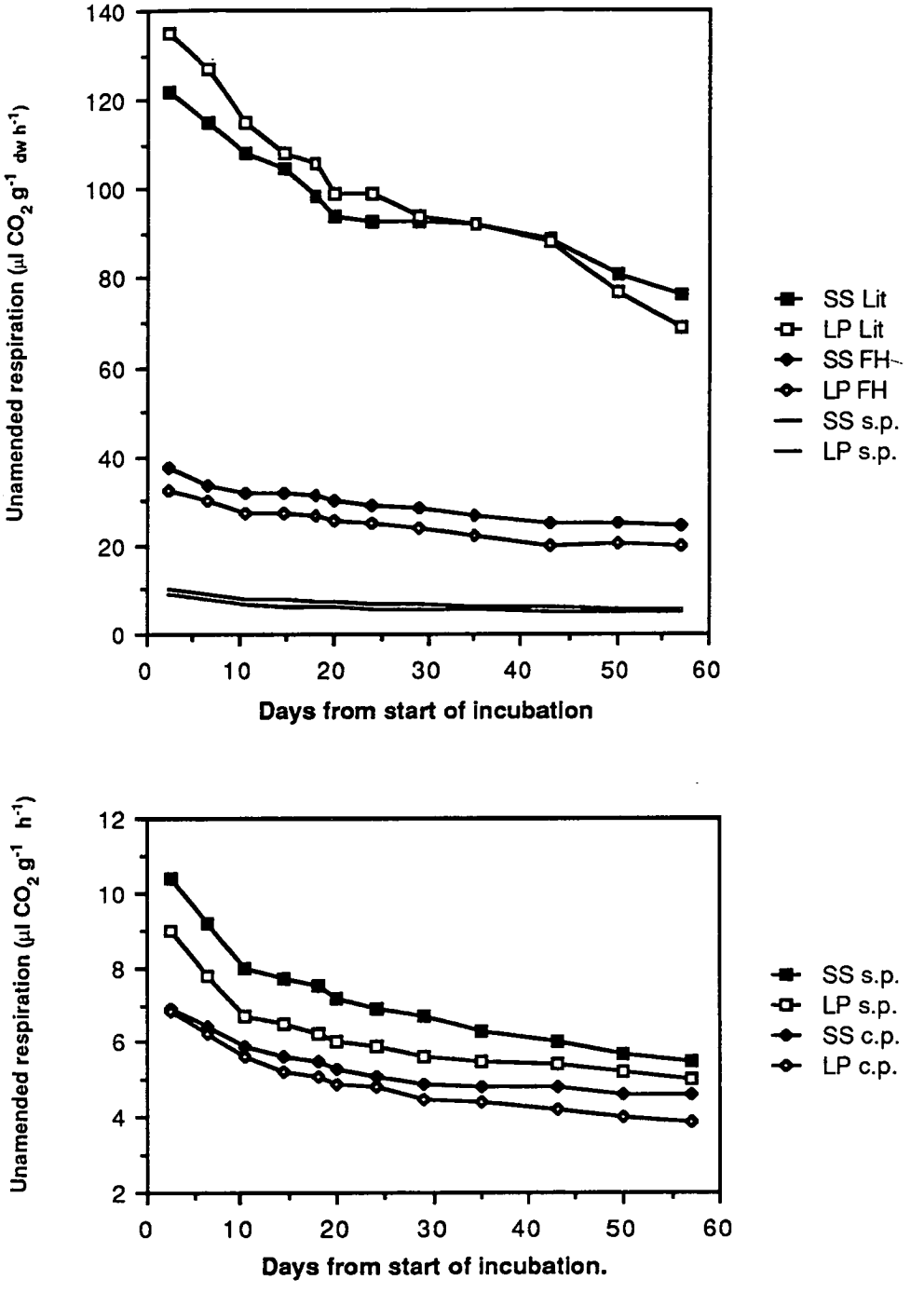


Figure 2. Profiles of respiratory activity during 60 day incubations. Abbreviations. Lit litter; FH forest FH material; s.p. underlying poorly humified *Sphagnum* peat layer; c.p. basal amorphous *Calluna* peat; SS Sitka spruce; LP lodgepole pine *s.p. results plotted in both graphs to indicate relative differences between substrates.*

After 30-40 days, respiration rates in all FH and peat substrates generally stabilized, though at this stage, rates in pure litter substrates were still usually in a curved phase of decline. All SS substrates respired at consistently higher rates than corresponding

LP ones, though as can be seen in the mean levels of net C mineralization, described later, the differences on separate sampling occasions were generally not significant.

Over the various incubation periods the highest mean CO_2 loss from a substrate was equivalent to 533 μg C day^{-1}, representing a weight loss of substrate over its incubation period of 6.4 - 8.0 % , given a C content of 0.40- 0.50.

Glucose levels - temporal variations.

Glucose concentrations over the March-December sampling period are listed in *Table 1* and shown graphically in *Figure 3*. In SS plots there was a close correspondence in the sequence of changes in all three substrates. The early December sampling showed a particularly synchronous and marked surge in glucose levels. While it may be of no direct relevance, it was noted that this surge occurred during a particularly mild early winter period. In LP plots, monthly changes in glucose levels were very much smaller in the peat substrates than in comparable SS substrates and in less obvious synchrony with overlying FH material.The *conditions* responsible for the temporal changes in glucose concentrations have not yet been identified.

Glucose levels - spatial variations.

Field moisture levels do not correlate in any meaningful way either temporally or spatially with glucose content. At least in FH material under Sitka spruce, *spatial variations* correlate well with the degree of comminution of litter, less comminuted samples having higher glucose concentrations. The evidence for this is presented in a later section.

In the peat horizons underlying LP material, the *spatial variation* in glucose concentration is generally fairly low, in the range 100-400 μg g^{-1} substrate (Table 1), and the concentrations in the samples of each substrate (n=7) tend not to reflect those measured in FH material removed from the same core. In the peat horizons underlying SS material however, spatial variations are much larger, up to 1600 μg g^{-1}, and the concentrations in samples tend to correlate well with those measured in FH material from the same core: 9 out of 12 r values were in excess of 0.75 and six of these were higher than 0.84. A good example of the relationship can be seen in *Figure 4*.

Key: Mean $_{s.d.}$ range

Table 1. Concentrations of free glucose and soluble carbohydrate in field samples collected at different times of the year. Abbreviations: **FH** forest FH layer; **s.p.** underlying raw *Sphagnum* peat layer; **c.p.** basal amorphous *Calluna* peat; **SS** Sitka spruce; **LP** lodgepole pine; **x** bulked sample; **n.a.** not available.

Units: $\mu g\ g^{-1}$ dw substrate.

	Free glucose (Glucose oxidase - peroxidase)							Carbohydrate (Anthrone)	Carbohydrate (Glucose oxidase-peroxidase)		
	MAR 17	APR 21	MAY 29	JUL 06	AUG 18	OCT 13	DEC 04	JUL 06	JUL 06	AUG 18	OCT 13
SS FH	680	1592_{235}	1156_{270}	1770_{49}	1177_{322}	1084_{385}	2649_{918}	3382_{710}	2351_{761}	1396_{323}	1527_{503}
	x	n.a.	758 - 1289	1611 - 2644	842 - 1666	534 - 1649	1449 - 4170	1594 - 3875	2175 - 4106	1053 - 1992	764 - 2227
LP FH	254	1029_{92}	1109_{573}	1490_{474}	987_{217}	826_{228}	935_{101}	3082_{392}	1998_{649}	1187_{183}	1208_{331}
	x	n.a	888 - 1640	600 - 2044	762 - 1365	564 - 1080	757 - 1046	2613 - 3656	868 - 2711	924 - 1353	754 - 1582
SS s.p.	874	1015_{57}	475_{155}	824_{318}	732_{549}	702_{316}	1516_{739}	2616_{473}	1586_{808}	949_{567}	1042_{407}
	x	n.a.	348 - 655	667 - 1525	558 - 1844	411 - 1207	790 - 2353	2206 - 2631	839 - 3121	624 - 2094	741 - 1676
LP s.p.	233	407_{16}	301_{83}	328_{161}	227_{88}	241_{142}	427_{174}	2249_{253}	652_{246}	455_{124}	439_{200}
	x	n.a.	159 - 341	236 - 629	188 - 369	102 - 389	233 - 684	1825 - 2531	413 - 877	282 - 647	207 - 593
SS c.p.	330	633_{38}	237_{84}	584_{390}	507_{178}	628_{245}	1368_{648}	2010_{503}	736_{550}	663_{222}	750_{232}
	x	n.a.	223 - 379	393 - 1372	213 - 707	472 - 1111	649 - 2196	1550 - 2825	575 - 1725	353 - 1024	602 - 1203
L.P.c.p.	220	264_{6}	195_{28}	204_{91}	150_{67}	129_{54}	261_{66}	1627_{588}	434_{87}	379_{92}	324_{53}
	x	n.a.	159 - 216	117 - 358	113 - 288	94 - 211	186 - 353	938 - 1844	375 - 588	289 - 541	269 - 389
Correlation with free glucose:								0.84	0.99	0.99	0.99

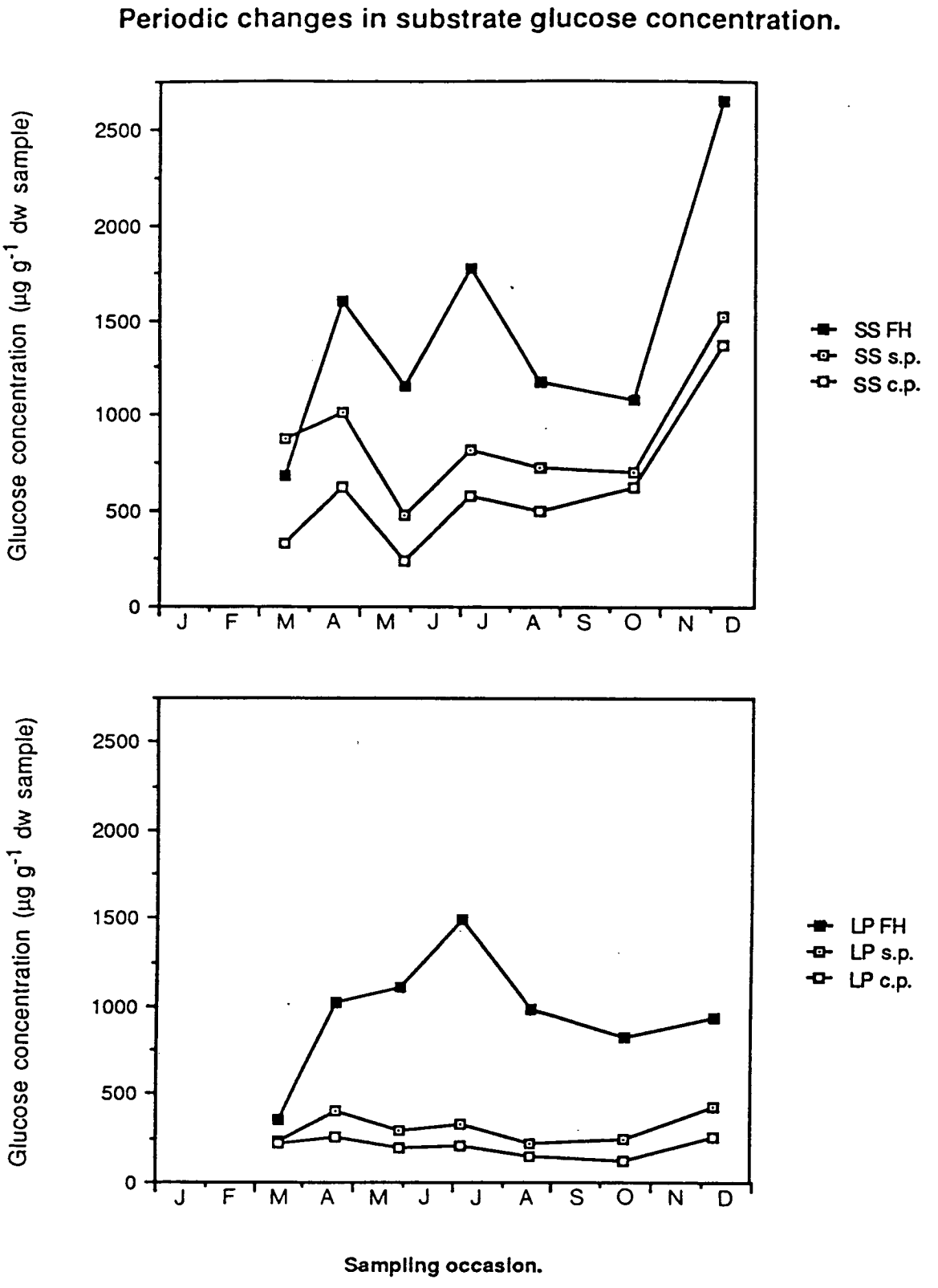


Figure 3. Periodic changes in free glucose concentration in material underlying Sitka spruce and lodgepole pine.
Refer to Table 1 for details.

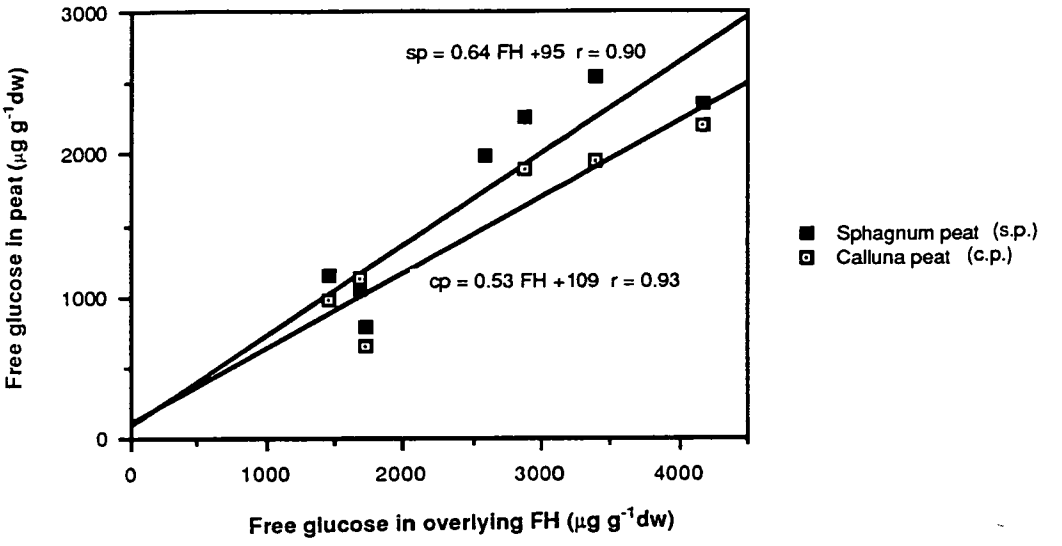


Fig 4, The relations between glucose concentrations in Sitka spruce FH samples and those in peat samples taken from the same core (Dec 04 sampling). Units: *μg glucose g^{-1} dw sample.*

This close correspondence between glucose concentration in SS FH samples and their underlying peat samples was also apparent in the mean temporal changes, noted above, which were more-or-less synchronous in all three substrates (Fig 3a). In contrast, the LP peats appear to be buffered from the temporal fluctuations occurring in overlying FH material (Fig 3b). A speculative explanation for this difference is that the FH layer is very much thinner in SS than LP and this results in the underlying peat horizons being much more exposed to changes occurring on the surface.Whether such changes are induced directly, say by environmental factors, or are indirect ones (e.g. leachates) reflecting changes in the FH layer, remains uncertain.

Glucose concentration *vs.* initial respiration.

An example of the relationship between initial glucose concentration and initial respiration (0-5 days) is shown in *Figure 5*. This indicates the general trend found on all sampling occasions, *viz.* a reasonably close relationship between the two variables (Table 2). As can be seen on *Figure 5*, however, the form which the relationship takes is more peculiar to each of the six substrates than to all of them taken together. A further interpretative complication is that while such relationships tend to remain good for the samples of each substrate at each sampling occasion (Table 2), the mean

periodic changes in one variable are not reflected in mean changes of the other (see Figures 3 and 9a,b). Between July and December for example, glucose concentrations undergo marked fluctuations (Fig 3), yet respiratory activity remains relatively constant (Fig 9a). A speculative explanation for this is that glucose concentration and respiratory activity are closely related only under relatively constant conditions, and that while this relative constancy exists for each substrate on each sampling occasion, it does not exist *between* substrate-types or *between* sampling occasions.

Table 2. Correlations between initial respiratory activity and initial respiration for each sampling occasion.

	MAY 29	JUL 06	AUG 18	OCT 13	DEC 04
SS FH	0.88	0.74	0.29	0.16	0.72
LP FH	0.93	0.75	0.77	0.70	0.73
SS s.p.	0.32	0.89	0.93	0.53	0.68
LP s.p.	0.80	0.88	0.63	0.94	0.80
SS c.p.	0.92	0.53	0.89	-0.40	0.90
LP c.p.	0.57	0.91	0.86	0.51	0.77

Glucose concentrations at the end of incubations.

At the end of incubation periods, mean glucose levels in substrates were between 0.20 and 0.60 times initial levels. The actual proportions were relatively consistent for each substrate over sequential sampling occasions. In FH material this proportion was usually between 0.25 and 0.40, while in LP material it was always higher, 0.45 - 0.60. The same trend was found in *Sphagnum* peat substrates where the corresponding ranges were 0.20 - 0.30 (for SS) and 0.40 - 0.50 (for LP). In the underlying *Calluna* peat the range was 0.15 - 0.25 for SS; a value for corresponding LP peat could not be calculated with sufficient accuracy because of the significance of extract autoabsorbance levels (usually equivalent to c. 40 - 60 μg glucose g^{-1} substrate) with respect to most end-of-incubation glucose levels (*c.* 50 - 150 μg g^{-1} substrate).

Glucose concentration as an index of labile carbon concentration.

No carbohydrate procedure is capable of measuring total carbohydrate in extracts (Doutre *et al.*, 1978). This is because colour reactions tend to be exclusive only to one sugar (e.g.glucose oxidase-peroxidase with glucose) or to a very limited class of sugars (e.g.

Composite plot:

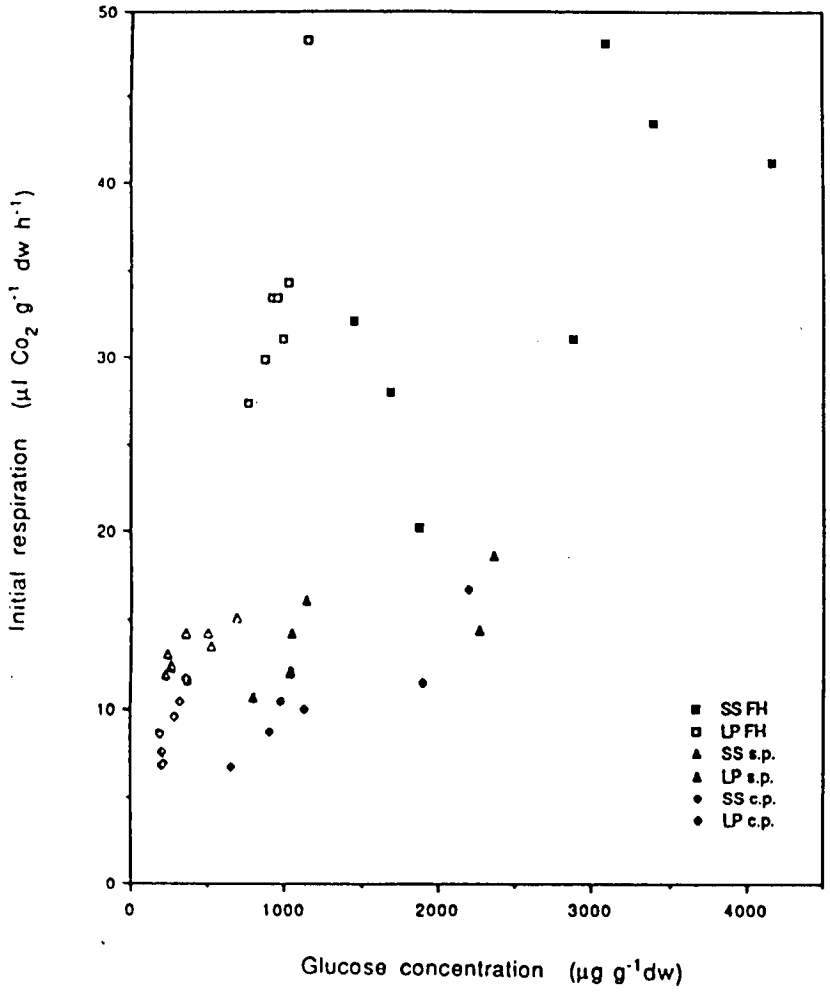
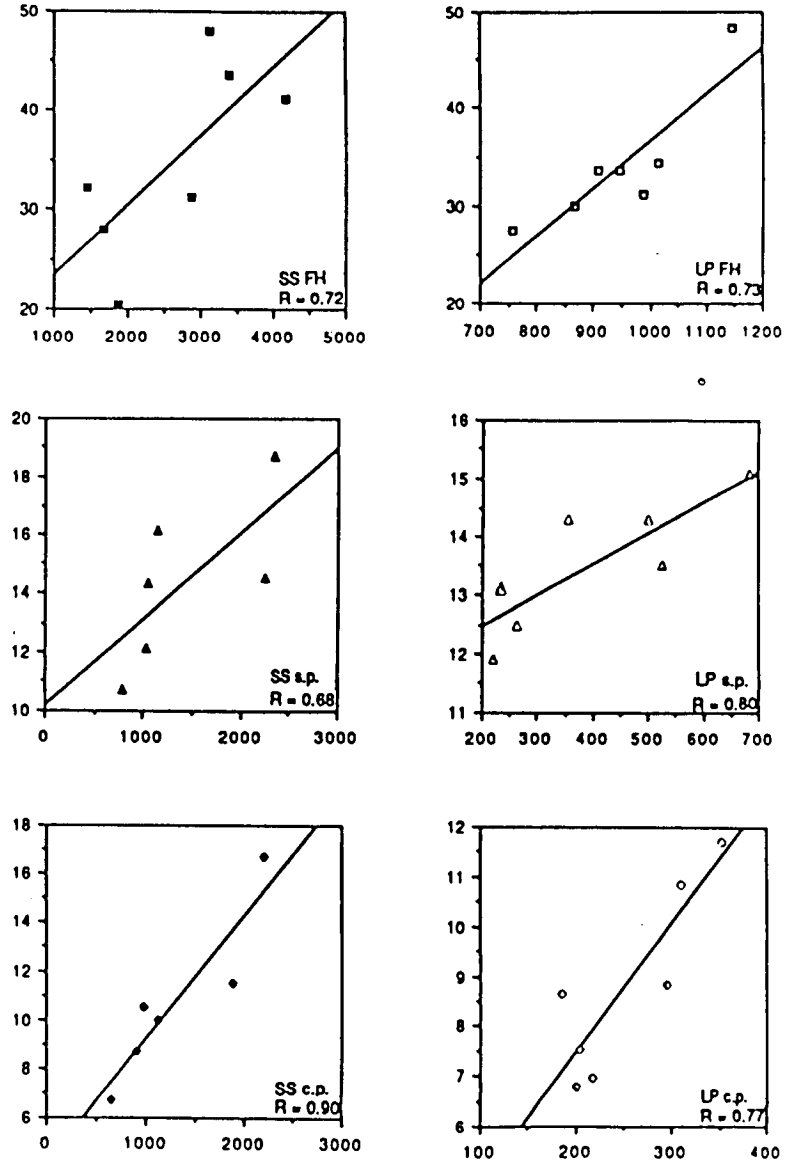


Figure 5. showing the relationship between initial respiration and free glucose concentration at sampling for each of the six substrates. Dec 04 Data. *Refer also to Table 2.*

Individual substrate plots:



anthrone with hexoses; Brink *et al.*, 1960; Ivarson and Sowden, 1962; Doutre *et al.*, 1978).

In this research, two methods were used to measure soluble carbohydrate. In one, *Method (a),* polysaccharide was hydrolysed by autoclaving in 0.5N HCl and after neutralization of mineral acid, glucose was measured enzymatically as described earlier (this thesis, page 25). Results represent *total free glucose plus soluble glucose polymers*, though here the term *soluble carbohydrate* is used for convenience. In the second method, *Method (b),* polysaccharide was hydrolysed in 20 N H_2SO_4 containing anthrone, as described, and hexose measured with reference to D-glucose standards. Results represent *total free hexose plus soluble hexose polymers*, though here the term *soluble carbohydrate* is again used for convenience (Doutre *et al.*, 1978).

The soluble carbohydrate results obtained in *Method (a)* correlated very closely with the free glucose concentrations measured in corresponding samples of all FH and peat substrates (Table 1b): correlation coefficients between the two sets of values were 0.99 for each data set (*viz.* 3 sampling occasions each with 42 samples). While these overall correlations were high, the *proportion* of carbohydrate accounted for by glucose varied between 0.30 and 0.98. This proportion was consistently lower in *the peat substrates under LP*, being 0.30 - 0.55, while in *all other substrates* it was nearly always between 0.65 and 0.85. An example of the close overall relationship between free glucose and carbohydrate measured in *Method (a)* is illustrated in *Figure 6*.

Because carbohydrate was measured by *Method (a)* on only 3 sampling occasions, it is not possible to consider seasonal variations, as was done for glucose (viz. Figure 4; Table 1), however given the close correspondence between the two measurements on these occasions it seems likely that seasonal changes in soluble carbohydrate are closely reflected in those described for free glucose.

With *Method (b)*, reproducibility was much worse than for *Method (a)* - up to 25% as opposed to <5% - and while it was found to be very good for highlighting large differences in hexose concentration, such as those shown in *Figure 7* and those described elsewhere (this thesis, chapter 4) it was considered unreliable for the smaller variations frequently present in FH and peat substrates.

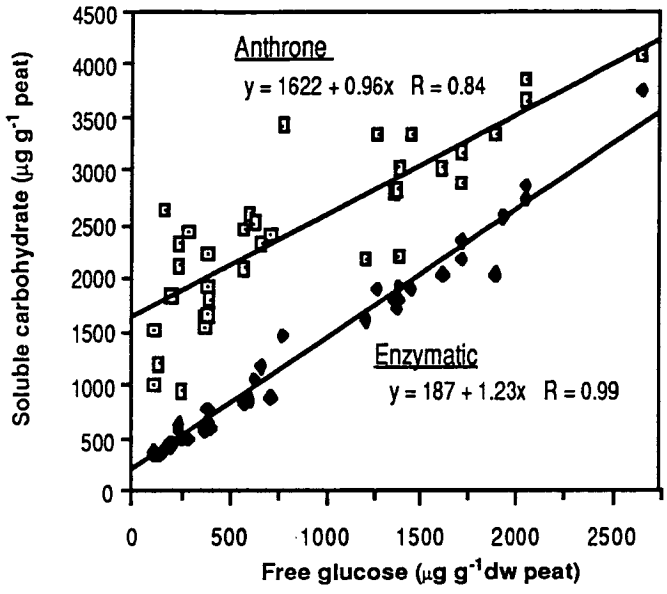


Figure 6. The relationship between free glucose, measured enzymatically and soluble carbohydrate, measured (a)enzymatically as glucose after hydrolysis: 0.5N HCl,121°C, 60 mins and (b)as hexose using anthrone. Jul 06 Data. ***Also see Table 1a.***

With FH material *Method (b)* gave mean readings 44-54% higher than *Method (a)* (Table 1). In the peat substrates, mean readings were up to 270% higher (Table 1b). In view of the possible interference from other dissolved organic material (see *Discussion*), the use of anthrone *for comparison of peat samples from different sources* was discontinued after the analysis of the Jul 06 material, though it continued to play an important role in other studies.

Accessibility of native labile carbon.

When a selection of samples from each substrate was amended with 2000 µg glucose g^{-1} substrate no net residue remained after 72 hrs. This rapid utilization rate of a glucose amendment together with the persistence of high concentrations of native glucose suggested *either* that native glucose was not readily accessible *or* that the release rate of glucose from its polymers exceeded its utilization rate from microbial metabolism.

When samples of unground and ground (0.5mm screen) recently-fallen litter were extracted in acetic acid, substantially higher glucose concentrations were present in the extract of ground material *e.g.*

ground	unground	
6720	2830	μg glucose g^{-1} substrate

The failure of acetic acid to extract all glucose from *unground* material indicated that microbes may not have ready access to a large proportion of a litter's labile carbon reserves in the early stages of decomposition.

The possible implications of such *labile carbon inaccessibility* for respiration were investigated by incubating 0.75 g samples of ground and unground litter at 80 % moisture in 280 ml winchester bottles. A thin more-or-less even spread of ground sample was obtained by placing bottles on a high-speed shaker for 10 minutes. Respiratory activity was measured at intervals over the next 60 days. The results are shown graphically in Fig 7. Over the initial 5 days CO_2 output was 52% higher from ground than from unground litter. Thereafter however, ground material respired at a much lower rate than unground material.

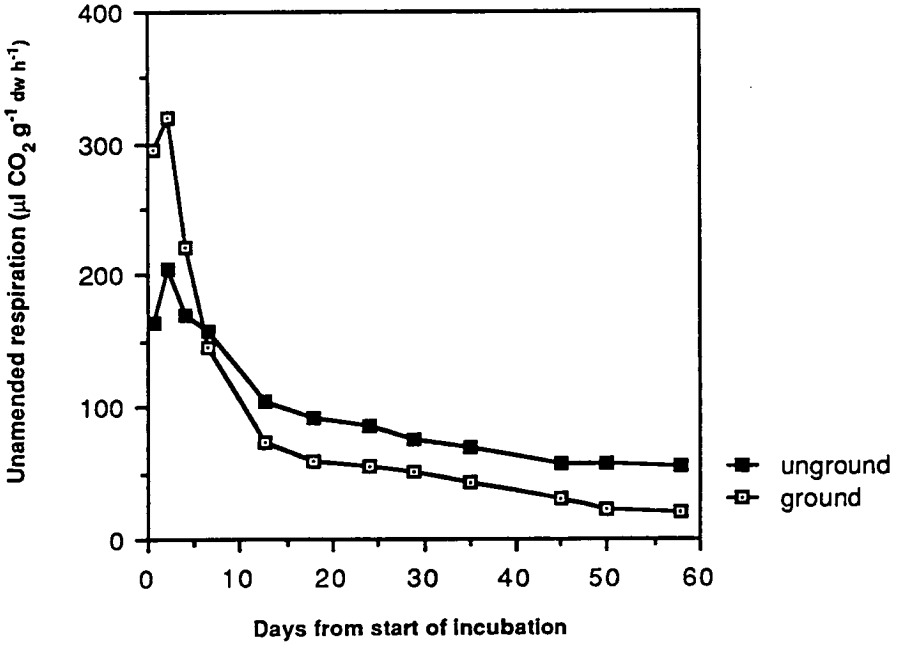


Figure 7. Respiratory activity from recently-fallen Sitka spruce needle litter in unground and ground (0.5 mm screen) states.

To investigate whether the rapid decrease in respiration might be related to substrate utilization, three replicate sub-samples of the *ground* material used above were removed at intervals during the incubation, for glucose and carbohydrate analysis. The results are shown graphically in *Figure 8*. Almost from the outset there was a precipitous fall in glucose and carbohydrate levels which were more-or-less in synchrony with the fall in respiratory levels.

Notably, the fall in glucose and soluble carbohydrate levels was not sustained towards zero concentration, but stabilized after c. 20 days at around 700 and 4000 μg g respectively. The correlation between soluble carbohydrate (measured using anthrone) and free glucose (measured enzymatically) over the incubation was r=0.99 *(y=3218+2.56x)*; and between soluble carbohydrate and respiration was r=0.99.

Taken together, the results of the above experiments indicate that a substrate's native labile carbon is very readily metabolized when accessible, *but that* in recently fallen litter, partial inaccessibility constrains activity in the earliest stages of decomposition.

The continued presence of glucose and soluble polysaccharide at concentrations well above zero indicates *either* that a particular fraction of a substrate's labile carbon is not readily available *or* that the overall release rate of glucose from enzyme-mediated hydrolysis of non-soluble carbohydrate exceeds its utilization rate through microbial metabolism.

Contribution of fresh litter and fine root to respiratory activity and glucose concentration.

The decision to take great care in removing fresh litter and fine roots from samples was vindicated by separate studies looking at the potential impact these might have on a substrate's respiratory and labile carbon levels.

Fresh litter and respiration. Initial respiratory levels of recently fallen litter from SS and LP plots always exceeded 120 μl CO_2 g^{-1} dw h^{-1} and were sometimes higher than 200 μl CO_2 g^{-1} dw h^{-1} (see Figures 2 and 6). In contrast, corresponding respiratory levels in FH substrates were usually between 20 and 40 μl CO_2 g^{-1} dw h^{-1}. The implications of this are that the presence of even small quantities of fresh litter in what

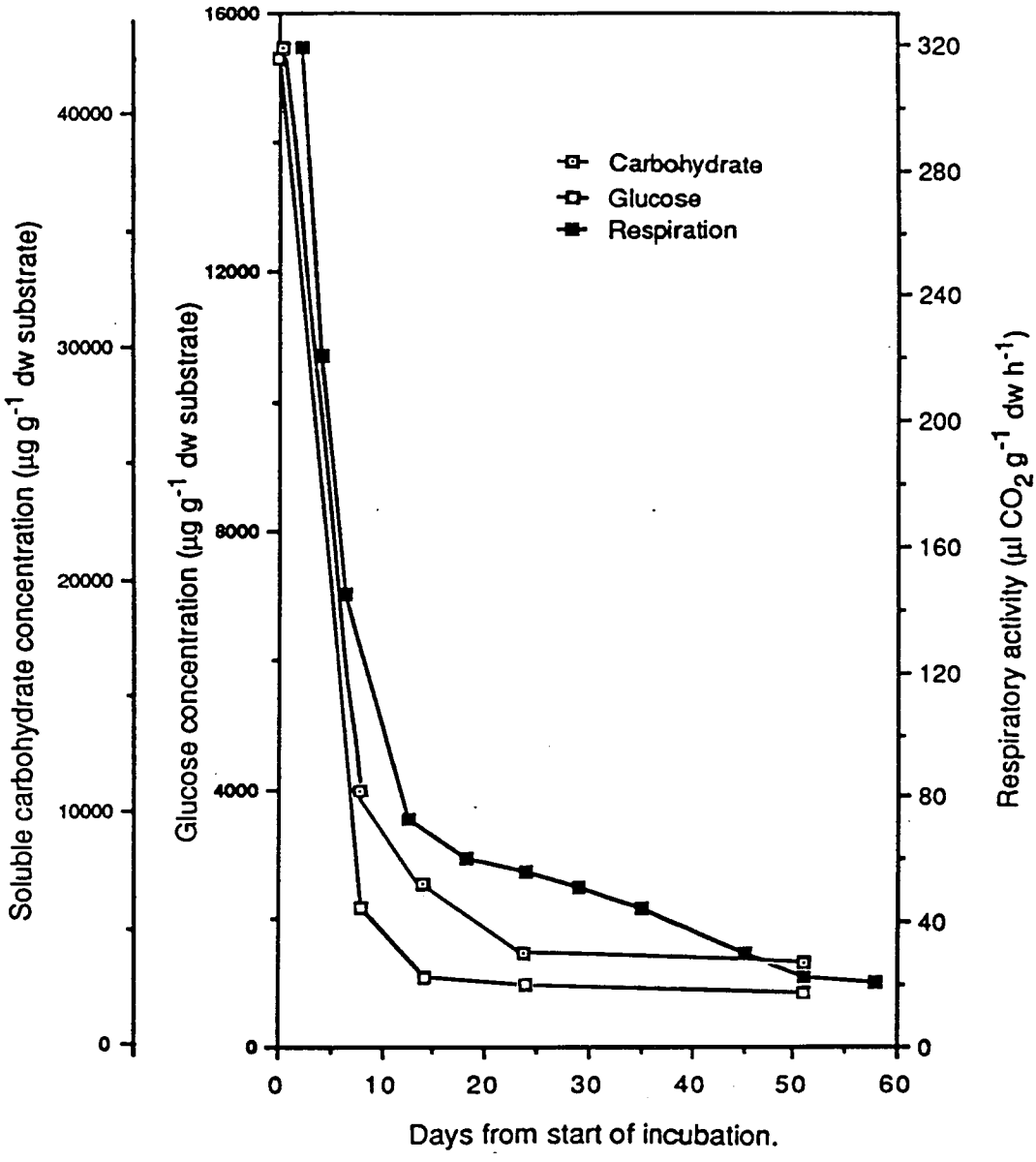


Figure 8. Changes in soluble carbohydrate and free glucose concentrations and in respiratory activity of ground Sitka spruce litter during incubation.

glucose measured enzymatically; soluble carbohydrate using anthrone.

is assumed to be an FH substrate could have a profound effect on respiratory rates. A low respiring FH substrate (20 µl CO_2 g^{-1} dw h^{-1}) contaminated with 10% of a high respiring litter (200 µl CO_2 g^{-1} dw h^{-1}) would have twice the respiratory rate of an uncontaminated one. To investigate the possible implications of this for the results reported here, 1g sub-samples of the *de-rooted FH substrates* used in the October measurements were examined under magnification and the following fractions were separated out and weighed:

(1) More-or-less intact needle litter.
(2) Needle litter fragments >5mm.
(3) Live root missed during earlier de-rooting.
(4) Residue.

Fresh litter was found to account for at most 1.5% of a sample's weight and on the basis of the respiratory values measured for such material (i.e. to 200 µl CO_2 g^{-1} dw h^{-1}) can be calculated to be quantitatively unimportant with respect to respiratory values obtained for FH material (*viz.* 0.015 X 200 µl CO_2 g^{-1} dw h^{-1}). In SS samples the partially comminuted litter (fraction 2) accounted for up to 40% of a sample's weight however, and the amount present was closely correlated to respiratory levels. In LP samples this was not the case: litter fragments were well comminuted: virtually all of a sample's weight was accounted for by fine residue (fraction 4).

Fine roots and respiration. Several procedures were followed in order to put the potential contribution of root respiration on substrate respiratory values into quantitative context. On two sampling occasions, a selection of substrate types were amended with the fresh-weight equivalent of 50-100 mg dw fresh fine root material which had replicate sub-samples removed for precise dry weight determination. These fine root amendments elevated CO_2 levels by up to 0.14 µl CO_2 mg^{-1} fine root h^{-1}. After 10 days, the contribution of root amendments to respiration was no longer detectable.

When 1g samples of the *de-rooted* substrates used in the October measurements were examined under magnification, as described in the previous section, the weight of fine root removed was generally under 10 mg root g^{-1} dw substrate, and the maximum level was 14 mg g^{-1}. In FH substrates such low levels would account for, at most,

10% of measured respiratory values (i.e. given the low respiration rate of 20 ml CO_2 g^{-1} dw h^{-1}). This, and the very poor correlation between the amount of root *contamination* removed under magnification (see above, fraction 3) and initial respiration of corresponding *contaminated* samples indicate a low-level contribution of fine roots to the respiratory values reported here.

In all *peat* substrates, levels of root contamination were barely detectable under magnification (< 5 mg g^{-1} substrate) and it is therefore assumed that they did not make a significant contribution to measured respiration rates.

These results lead to the conclusion that the substrate respiratory values reported here are almost entirely attributable to decomposition activities, and at least in the case of FH material under SS are determined by measureable substrate characteristics: here, the degree of comminution (as measured using the categories described) is presumably a measure of the state of decomposition, and was closely correlated with respiration rate.

Fresh litter and glucose concentrations. Fresh litter is a very rich source of labile carbon. Up to 7474 µg glucose g^{-1} have been extracted from ground Sitka spruce material. Almost certainly however, this is only partially accessible to microbes at the outset of decomposition. Indirect evidence for this comes from the large differences between the quantity of glucose extracted from fresh ground and unground litter (2% CH_3COOH, 16 hrs) and from the increasing quantities of glucose that can be extracted from fresh unground litter with extraction times greater than 16 hrs.

The practical implications of these findings are as follows: (1) since the amount of labile carbon extracted from fresh unground litter over 16 hrs is a relatively small proportion of its total content, such extractions should not be used on substrates of this type without due regard to the implications, and (2) where an FH sample is thought to contain fresh litter, the importance of the litter should be put into quantitative context.

Because of the low levels of fresh litter removed from FH substrates (up to 1.5% substrate wt.), fresh litter is not thought to have contributed significantly to the glucose concentrations of FH material reported here.

Fine roots and glucose concentrations. Like fresh litter, fine roots are a lucrative

source of labile carbon. Samples of SS and LP fine root were extracted on many of the sampling periods and concentrations of glucose in the respective ranges 6.03 - 12.3 and 3.01 - 7.76 μg mg^{-1} root were measured. The implications of these results are the presence of even small quantities of roots in samples, say 10-100 mg g^{-1} substrate, could could have a major impact on substrate glucose measurements.

When root contamination levels were measured in FH substrates, levels were occasionally as high as 14 mg g^{-1} substrate, and on this basis it could be calculated that roots might contribute up to 172 μg glucose g^{-1} substrate (*i.e* 14X12.3). Contamination levels were generally much lower however (2-8 mg root g^{-1} substrate), and seem unlikely therefore to have made an important contribution to measured respiratory values. This is also the case for peat samples, where the levels of contamination were barely detectable (<5mg g^{-1} substrate).

The ability of the extraction procedure to extract such relatively large quantities of glucose from fine root fragments, highlights the importance of rigorous root removal from substrates as well as follow-up procedures on a representative number of samples to ensure that the potential impact of root contamination on the results has been accounted for.

N mineralization and C:N mineralization ratios during incubations.

For each sampling occasion net C mineralization rates in the samples of each substrate type (n=7) provide no clue to net N mineralization rates. Mean values for each sampling occasion can be seen in *Table 3,* and this information is presented graphically in Figure 9. Almost without exception, the results show that substrates removed in the summer mineralize less N during incubation than those removed in spring and winter. Over the March-August period, N mineralization rates in FH substrates of SS and LP fall by 56 and 77% respectively. Changes in C mineralization are smaller and less consistent, and in LP material the overall result is an increase in the C:N mineralization ratio from 20 to 100. In this case, the very low seasonal changes in C mineralization suggest that overall changes in substrate quality cannot be invoked to account for the N mineralization changes.

N mineralization rates in all SS substrates were consistently higher than corresponding LP substrates. Given the apparent uniformity of the peat horizons between the two

Table 3. C and N mineralization values and C:N mineralization ratios during 60-day incubations of samples collected on different occasions. Incubations carried out at 84% moisture and 20°C. Abbreviations: **FH** forest FH layer; **s.p.** underlying raw *Sphagnum* peat layer; **c.p.** basal amorphous *Calluna* peat; **SS** Sitka spruce; **LP** lodgepole pine; **x** bulked sample. Data presented graphically in Figure 9.

Key: $\frac{\text{Mean C mineralization}_{s.d.}}{\text{Mean N mineralization}_{s.d.}}$ C:N mineralization ratio

	MAR 17	APR 21	MAY 26	JUL 06	AUG 18	OCT 13	DEC 04
SS FH	$\frac{381_{x}}{19.3_{x}}$ 20	$\frac{533_{97}}{14.1_{5.1}}$ 38	$\frac{464_{106}}{15.0_{3.4}}$ 31	$\frac{326_{60}}{10.4_{3.2}}$ 31	$\frac{365_{86}}{8.45_{0.67}}$ 43	$\frac{301_{84}}{8.62_{2.49}}$ 35	$\frac{284_{73}}{8.06_{3.54}}$ 35
LP FH	$\frac{159_{x}}{13.3_{x}}$ 12	$\frac{374_{142}}{7.09_{2.31}}$ 53	$\frac{361_{159}}{5.45_{1.80}}$ 66	$\frac{313_{93}}{3.49_{2.86}}$ 90	$\frac{310_{48}}{3.11_{2.30}}$ 100	$\frac{262_{63}}{5.56_{1.80}}$ 47	$\frac{261_{40}}{4.62_{1.83}}$ 57
SS s.p.	$\frac{159_{x}}{6.71_{x}}$ 24	$\frac{374_{142}}{6.72_{1.98}}$ 22	$\frac{362_{159}}{5.00_{2.35}}$ 28	$\frac{313_{93}}{3.89_{0.89}}$ 25	$\frac{89_{17}}{3.63_{0.72}}$ 24	$\frac{74_{16}}{2.75_{0.61}}$ 27	$\frac{120_{27}}{6.98_{1.83}}$ 16
LP s.p.	$\frac{156_{x}}{3.44_{x}}$ 46	$\frac{90_{31}}{2.66_{0.68}}$ 34	$\frac{118_{23}}{3.28_{1.16}}$ 36	$\frac{77_{22}}{3.05_{1.38}}$ 34	$\frac{76_{6}}{2.13_{0.20}}$ 36	$\frac{72_{32}}{2.28_{0.97}}$ 32	$\frac{89_{10}}{5.44_{0.74}}$ 16
SS c.p.		$\frac{88_{17}}{2.78_{0.71}}$ 32	$\frac{89_{20}}{2.05_{0.54}}$ 43	$\frac{68_{19}}{2.00_{0.43}}$ 34	$\frac{69_{14}}{1.59_{0.46}}$ 43	$\frac{66_{10}}{1.85_{0.70}}$ 36	$\frac{94_{27}}{5.27_{1.29}}$ 18
LP c.p.		$\frac{67_{15}}{2.19_{0.47}}$ 30	$\frac{80_{17}}{2.05_{1.05}}$ 39	$\frac{51_{17}}{1.74_{1.03}}$ 29	$\frac{61_{13}}{1.53_{0.37}}$ 40	$\frac{44_{13}}{1.92_{0.32}}$ 23	$\frac{67_{13}}{4.45_{0.53}}$ 15

Units for C and N mineralization: $\mu g\ g^{-1}$ sample day^{-1}

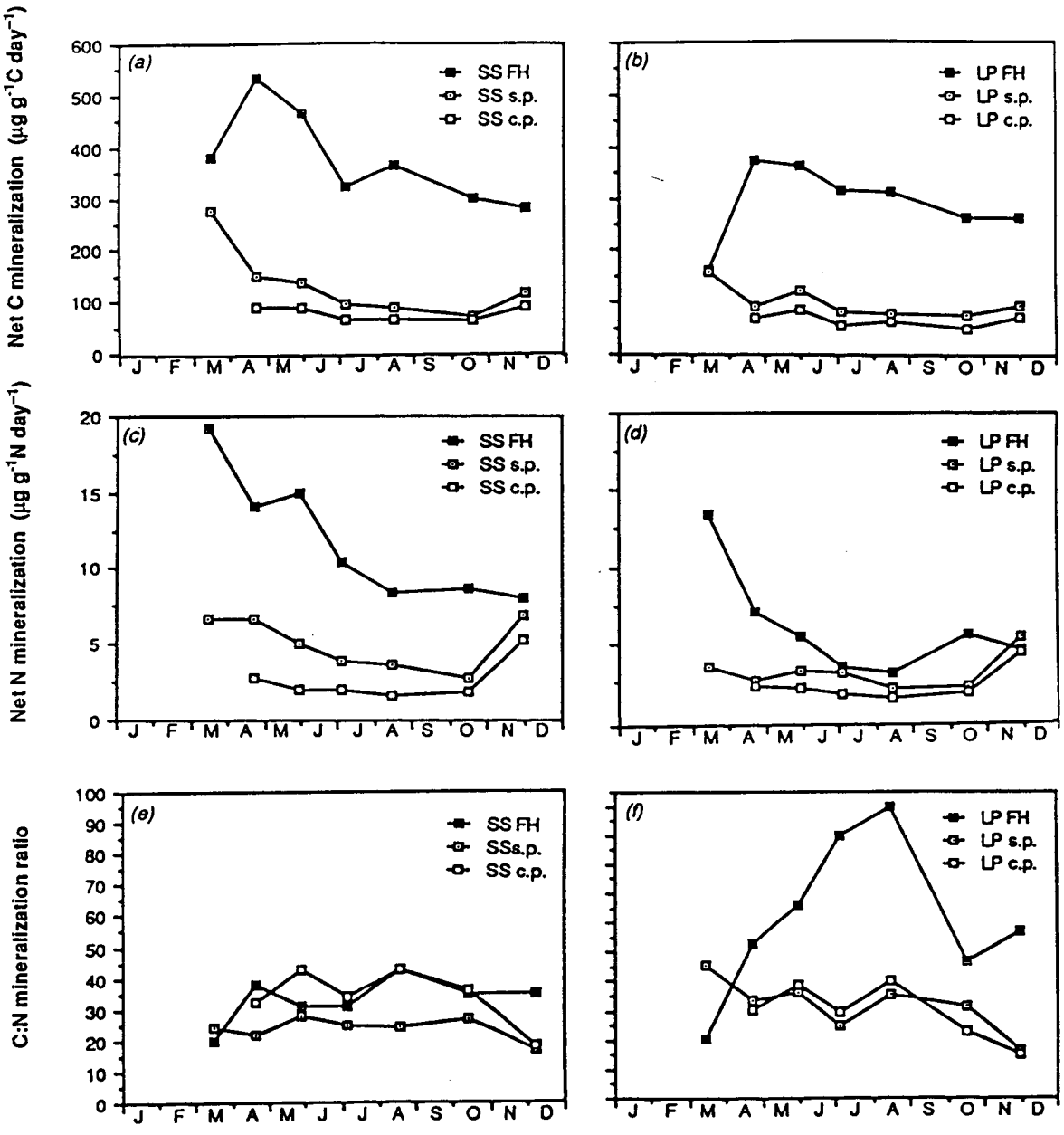


Figure 9. Mean C mineralization, mean N mineralization and mean C:N mineralization ratios during incubations of samples collected on different occasions. Incubations carried out for 60 days at 84% moisture and 20°C. Abbreviations: **FH** composite forest FH material; **s.p.** underlying poorly humified *Sphagnum* peat; **c.p.** basal amorphous *Calluna* peat. Refer to Table 3 for further details.

plots, the large N mineralization difference in the March-May period is particularly notable. Another consistent trend is the marked surge in both C and N mineralization in all four *peat* substrates during the period October-December. This surge contrasts with the small changes in C and N mineralization rates over this period in overlying FH materials.

NO_3-N was detected only in very low concentrations at the end of incubations and in all cases NH_4-N accounted for more than 95% of mineral-N measured.

DISCUSSION.

All the results have been described in the form *x* g^{-1} *dw substrate* where *x* is the variable measured. In terms of *x* m^{-2} *forest floor* the relative differences between species might frequently be reversed, but such conversions have been avoided. The aim here has been to consider substrate quality variations and the possible implications of these for some microbial processes. In view of the absence of plot replicates, the restricted areas of sampling (flat areas, not ridges or furrows) and the short sampling period (9 mths) the results should not be seen to warrant comparison at a forest-plot level. Here, where substrates have been compared with references to species (*viz.* SS, LP), the latter have been used only as labels and no implication of differences at forest-plot level is intended.

The respiratory results have shown a high correlation between respiration at the outset of incubations and respiration after c. 8 wks (days 55-60). When samples were amended with up to 100 mg fresh fine root, the contribution to respiration was no longer detectable after c. 2 wks. In the light of these observations and the calculated values showing the low respiratory activities that can be expected from the levels of root contamination measured in samples, it can be assumed that the substrate respiratory activities reported here are almost entirely attributable to decomposition processes.

There are a combination of factors which could account for the initial decline in respiratory activity during incubations - the depletion of labile carbon, the mortality of mycorrhizal hyphae and disturbance from handling - and it has not been possible in this research to extricate one factor from another.

In this and in related investigations (this thesis, chapters 3 and 4), there was no general relationship between a sample's respiratory rate during an incubation and its N release rate, even among samples of apparently uniform substrates: in this case, among samples of each of the two peats. This suggests that even in substrates in a late stage of decomposition, trends in mineral-N release are not synchronized with the overall trends in CO_2 release. In heterogeneous substrates, where C and N are held in diverse fractions of varying lability a general relationship is hardly to be expected, and the absence of one casts doubt on the measurement of CO_2 release in such substrates as an aid to understanding the conditions which favour mineral-N release.

The fairly constant C mineralization values shown in *Figure 9* (April-December), contrasting with the variable N mineralization values, may be further evidence that gross substrate quality (as measured by C mineralization) cannot be invoked to explain N release in samples: in FH material under both SS and LP substantially more N is mineralized in winter than in summer (Figures 9a and b), despite no change in incubation conditions (84% moisture; 20°C). Under LP there appears to be little change in substrate quality, as indicated by net C mineralization (Figure 9d Apr-Dec) and this results in a large mid-summer peak in C:N mineralization ratios (Fig. 9f). In other research, seasonal changes in microbial activity (Nicholas *et al.*, 1965; Biederbeck and Campbell, 1971; Flanagan and Veum, 1974; Campbell and Biederbeck, 1976; Söderström, 1979; Clarholm and Rosswall, 1980; Bååth and Söderström, 1982; Lundgren and Söderström, 1983) and net N mineralization (Campbell and Biederbeck, 1972; Van den Driessche, 1977; Nadelhoffer *et al.*, 1984) have been attributed to variations in moisture and/or temperature conditions. In this research, given the constant temperature and moisture conditions of incubations, the results could suggest that one or more other variables play an important role in regulating N release.

While the enzymatic assay appears to be ideal for *free glucose* measurement, neither the enzymatic assay (as applied to hydrolysed extracts) nor the anthrone assay are considered satisfactory for future comparative measurements *of soluble carbohydrate* in different field substrates. The enzymatic assay has two main drawbacks: firstly it measures only glucose plus glucose polymers and while these are usually reported to account for a high proportion of carbohydrate in soils and litters (Cheshire and Mundie 1965, 1966; Greenland and Oades, 1975), the concentrations of other sugars and their polymers can be substantial (e.g. Parsons and Tinsley, 1961; Sowden and Ivarson,

1962; Greenland and Oades, 1975). A second and more fundamental drawback is that if possible, it is better to have a completely independent method for measuring soluble carbohydrate than the one used for the free glucose fraction. The anthrone procedure, however, while presumably giving a better indication of *total* soluble carbohydrate, involves intense hydrolytic conditions which may result in the release of carbohydrate contained as an integral part of dissolved humic substances (see Cheshire and Mundie, 1966; Haworth, 1971; Cheshire *et al.*, 1975; Cheshire, 1977). This means that it is not possible to be certain whether the high concentrations of carbohydrate measured in peat samples relate to high concentrations of soluble humic material, or whether they actually indicate a higher availability of carbohydrates other than free glucose.

In this research programme, the poor reproducibility of the anthrone procedure noted by others (Doutre *et al.*, 1978) could not be overcome. This and other procedural aspects of the this study have been discussed in greater detail elsewhere (this thesis, chapter 2).

The results for substrate glucose concentrations reported here are not directly comparable to those reported elsewhere, firstly because of the different extraction procedures used, and secondly and perhaps more crucially because those measured by other researchers appear to have been done on a *one-off* basis and therefore give no clue to temporal or spatial variations. As shown earlier, these can be considerable (Table 1): in the December collection for example, there was almost a 3-fold *spatial* variation in glucose concentrations in FH material under SS (1449-4170 $\mu g\ g^{-1}$ substrate) while mean *temporal* variation between October and December was 2.4-fold (from 1084 to 2649 $\mu g\ g^{-1}$ substrate).

Using water as an extractant, Alsvaker and Michelson (1957) reported glucose concentrations of 2200 $\mu g\ g^{-1}$ in the F horizon of a pine forest floor. In an extension of this work Grov(1963) measured levels of 1890 and 1820 $\mu g\ g^{-1}$ in H and A horizons; glucose accounted for 79 and 83% respectively of the total free sugar content. These concentrations of glucose fall into the range measured in pine FH material during the course of this research programme *viz.* 564-2044 $\mu g\ g^{-1}$ (Table 1, line 2).

Using 80% ethanol as an extractant, Gupta and Sowden (1963) reported glucose concentrations of 18 and 30 $\mu g\ g^{-1}$ in two peats and 100 $\mu g\ g^{-1}$ in the organic horizon

of a podzol. In the peats no other free sugars were detected. Based on the results of trials carried out as part of this research, however, it seems likely that their extraction procedure - *a 2hr extraction with occasional shaking* - was inadequate for extracting most of the glucose from samples. They also found a reduction of up to 50% glucose concentration in samples which had been air-dried prior to extraction and noted that this might indicate more utilization by microbes during drying than breakdown by enzymes. An alternative possibility, however, is that a smaller proportion of glucose was extracted from their air-dried samples over the 2 hr extraction period, and that with an overnight extraction no such difference would have been apparent.

Here and in related research (this thesis, chapters 3 and 4) glucose concentrations in a variety of unplanted peats have ranged from 50 to 148 $\mu g\ g^{-1}$ dw peat, while in the peats underlying the FH horizons of SS and LP plots concentrations ranged from 94 to 2353 $\mu g\ g^{-1}$ dw peat (Table 1).

A prospective explanation for the continued presence of relatively high concentrations of soluble carbohydrate, including free glucose, even after material has been ground (0.5mm screen) and incubated for many weeks, is that extracellular enzymes are active at microsites out of the immediate reach of their source microorganisms and this results in the presence of a reserve of glucose which influences its utilization rate (Swift, 1976, 1982). Indirect evidence that this is so can be seen in the increases in soluble carbohydrate measured by Frankland (1969) long (1 year) after decomposition had commenced. The production of extracellular polysaccharidases *in vitro* has been demonstrated and its implications for decomposition have been considered in the light of recent photomicrographic (Bravery, 1971) and biochemical investigations (Green, 1980; Levy and Dickinson, 1981; Levy, 1982; Montgomery, 1982): notably, the enzymes of brown-rot fungi appear capable of diffusing considerable distances from their source hyphae and this has been attributed to the absence of mucilage coating on brown-rot hyphae which in other fungi appear to be responsible for enzyme retention (Green,1980; Green *et al.*, 1980; Levy and Dickinson, 1981; Jones, 1982). The production of excess sugar at a distance from the site of uptake by brown-rot fungi has been suggested for wood decomposition (Cowling, 1961; Cowling and Brown, 1969). The ecological advantage of free enzymes may relate to increased diffusion of the products of depolymerisation or to a growth response from scouting hyphae which these could invoke (Burns, 1978). Alternatively, the presence of labile catabolites associated with extracellular enzyme activity of brown-rot hyphae appears to stimulate

the growth of secondary organisms and this may result in a net acceleration of decomposition (Blanchette and Shaw, 1978; Swift, 1982). For some brown rot fungi, the availability of labile carbohydrate also appears to be essential for the digestion of crystalline cellulose (Higley 1977, 1978; Montgomery, 1982). The stability of free enzymes which are in contact with some soil components may be considerable (Ladd, 1978).

A correlation between soluble carbohydrate concentration and sample respiration was reported by Spalding (1977) who collected 84 coniferous litter samples from diverse mixed-species forests in Oregon, USA. He measured soluble carbohydrate using anthrone (Spiro, 1966) after extraction of *ground* material with dilute potassium acetate, pH 5, and measured the respiration of *unground* material from the CO_2 absorbed in alkali over 4 days. Soluble carbohydrate varied between c. 2400 and 10,100 $\mu g\ g^{-1}dw$ and respiration between 160 and 896 $\mu l\ CO_2\ g^{-1}\ dw\ h^{-1}$. The correlation between the two variables was $r=0.74$, $p<0.01$. In the light of the research reported in this chapter (see *Accessibility of native labile carbon*) it is conceivable that an even closer relationship might have been detected if it were not for probable *differential inaccessibility of labile carbon* among the various litter samples: in this respect, glucose extracted from *unground* material might have provided a better index of accessibility to microbes. Notably, Spalding also obtained good correlations between respiration and amylase activity ($r=0.71$, $p<0.01$) and respiration and cellulase activity ($r=0.58$, $p<0.01$) and suggested a possible causal relationship between these enzyme activities and the concentrations of free sugars.

A correlation between soluble carbohydrate concentration and N mineralization was reported by Jenkinson(1968) who extracted 54 *mineral* soils with 0.1N barium hydroxide and measured soluble carbohydrate using anthrone. Concentrations ranged between 60 and 770 $\mu g\ g^{-1}$ soil and were closely correlated to N mineralization rates during 21 day incubations ($r=0.7$, $p<0.01$). A close positive relationship between the amount of carbohydrate in soil extracts and the amount of N mineralized during laboratory incubations was also reported by Verstraeten *et al.*(1970).

Finally, it should be pointed out that while there is general recognition that free sugars are most common in cool climatic conditions where surface litter is abundant (Gupta,1967; Greenland and Oades, 1975; Lowe, 1978; Stevenson, 1982), most

studies have dealt only with their importance in the earliest stages of decomposition. The results reported here strongly suggest that the implications of the presence and concentration of free sugars in the later stages of litter decomposition warrants further experimental and critical consideration.

CONCLUDING REMARKS.

The main objective of the research reported here has been to test some rapid, simple and sensitive procedures for measuring labile carbon and respiratory activity on a range of forest floor materials. A secondary objective has been to assess the potential value of using these techniques in a comparative study of forest floor materials under different species.

In addition, the research programme was devised in such a way that seasonal and spatial variations in net C and N mineralization might be found which could be attributable to variables other than temperature and moisture. Such variables, if significant, could open up the longer-term prospect of manipulating decomposition with a view to optimizing nutrient release processes within the prevailing climatic constraints.

In view of the considerable time-input usually needed for field-based research, and the low-resolution quality of the information which often emerges, further such work would probably benefit from interim *experimental* research aimed at identifying potential factors responsible for the temporal changes in glucose concentration and net N mineralization. As Spalding(1977) points out, material *must be perturbed and changes in suspected causal variables observed* if a better understanding of decomposition is to be achieved. In order to assess the potential value of measuring glucose concentrations in decomposing field substrates, the procedures used here probably need to be complemented by assay procedures which measure specific enzyme activities. This should allow more appropriate questions to be asked and a more effective research strategy to be devised.

In summary, this research has shown that:

(1) The glucose concentrations in samples of *de-rooted partially decomposed* substrates correlate well with their respiratory activities though the relationship

between *mean substrate glucose content* and *mean respiration* is not constant through the changing seasons.

(2) The respiratory activities in the initial few days of an incubation of samples of *de-rooted partially decomposed* substrates are closely correlated with their longer-term (>c.8 wks.) activities. This suggests that initial activity may be a very good index of substrate quality.

(3) With fresh litter, an over-night extraction in 2% CH_3COOH results in much lower extraction of glucose than from corresponding ground material. Such apparent substrate *inaccessibility to extractant*, presumably due to the structural integrity and high wax content of fresh material, appears to reflect initial *inaccessibility to microbes:* unground litter has lower initial respiration rates and lower glucose and soluble polysaccharide utilization rates than corresponding ground material.

(4) Marked seasonal fluctuations in substrate glucose concentration occur. The possibility that peak glucose concentrations correlate with other observed phenomena (*e.g.* mushroom blooms) warrants investigation and with further research such fluctuations may also provide a useful index of one or more conditions which impact upon decomposition processes.

(5) Where the FH mantle is shallow (in this case in SS plots), the glucose content and respiratory activities in underlying peat usually correlate well with those in FH material *directly* above. This, combined with the lower *temporal and spatial variability* and the lower *absolute* values in respiratory activity, glucose content and N mineralization under a large FH layer (in this case in LP plots), could point to a general phenomenon: the thicker the FH layer the more insulated the underlying peat becomes from the overlying forest and the conditions which operate therein.

This research programme, together with others (this thesis, chapters 2, 3, 4; Morgan, in prep.) has provided the oportunity for the intensive testing of a single extractant capable of the simultanous extraction of labile carbon, NO_3-N, NH_4-N, P, K, Ca and Mg. The use of this in combination with the sensitive automated procedure for extractant-glucose measurement should provide an appropriate experimental setting for future integrated studies dealing with the role of labile carbon in mineral nutrient dynamics. Where measurement of enzyme activities are required in addition to labile-C and mineral-N levels, the use of an acetate extractant of higher pH may have to be considered.

CHAPTER 6

(ABIOTIC STUDY)

INFLUENCE OF SOME ABIOTIC FACTORS ON C AND N RELEASE FROM AN ACID OLIGOTROPHIC PEAT.

CHAPTER ABSTRACT.

In order to study the effects of mineral nutrient amendments on decomposition rates, it is necessary to take account of any associated changes in pH. Integrated studies which looked at pH changes as well as mineral nutrient amendments, have shown that mineral N, P, K, Ca or Mg amendments have no positive effects on CO_2 release from an acid oligotrophic peat, pH 3.5. The addition of dilute alkali solutions (0.01N), sufficient to increase pH by 1 unit to pH 4.5, had a large initial influence on CO_2 release, perhaps as a result of initial solubilisation of C components, but this influence greatly diminished over subsequent weeks: after six weeks, for example, CO_2 release was only 10% higher than controls. This pH increase resulted in immobilization of native mineral N over the duration of incubations (6 weeks). The addition of mineral salts at or below common field fertilization rates reduced pH and resulted in immediate reductions in CO_2 release. These reductions showed no sign of diminishing with time. A $CaSO_4$ addition, for example, which reduced pH by 0.4 pH units, reduced CO_2 release by 28% and this reduction was sustained throughout the 6-week incubation period. NH_4NO_3 and less notably KCl appeared to reduce CO_2 release more than was expected from their impact on pH. While the decomposition rates of this acid peat are unchanged by PO_4-P and K additions *per se* and are *either* unchanged *or* suppressed by NH_4-N additions *per se*, the presence of a plentiful supply of all three nutrients greatly enhances the decomposition rate of a labile C amendment. An observation which may have wider implications for decomposition studies is that in the absence of a plentiful supply of these nutrients, much more added labile C is incorporated into the pool of refractory substrate residues.

INTRODUCTION.

During the past three years, about 1400 samples have been incubated for periods of up to 120 days, as part of an overall study on the factors that influence C and N release. Those studies relating to the effect of plants have already been described. Some additional studies which may help to put the potential role of plants into a more meaningful context are briefly reported below. These aimed to assess the effects of **pH adjustments, mineral nutrient amendments, and glucose amendments** on C and N release. The mixed *Sphagnum-Calluna* peat used in the microcosm experiment was used in all of these investigations. It had a pH of 3.5, a *total* nitrogen concentration of 1.12% (Page 44, Table 6), and extractable mineral N, P and K concentrations (2% CH_3COOH, 16 hrs) of 470, 36 and 167 $\mu g\ g^{-1}$ dw respectively. Mineral N was present almost totally (>95%) as NH_4-N. Each treatment comprised three replicate 1.5 g subsamples and these were incubated at 20°C and 84% moisture content. The release of C and N over 42 days was measured using the procedures already described (chapters 2 and 3). Replicate variation in C and N release was generally very low (<5%) and unless noted otherwise, all reported treatment differences are significant at p=0.01. Great care was taken in measuring pH in order to sensitively reflect the small changes imposed by amendments. Measurements were made using an additional three replicate subsamples at a 1:25 dw:water ratio.

METHODS, RESULTS AND DISCUSSION.

pH adjustments. The pH in seven separate treatments was increased in increments up to a maximum of 1.0 unit, to pH 4.5, using 0.01 N NaOH. Addition rates were determined in preliminary investigations. A pH increase of 1.0 unit required an addition of 0.15 mmoles NaOH g^{-1} dw peat. In the first week of the incubation, CO_2 release rates increased in close correspondence with the induced increases in pH (Figure 1). At the highest pH, *pH 4.5*, release was 48% higher than controls (Figure 1). This amounted to 252 μg excess carbon released over the first week with respect to controls. In subsequent weeks, however, the differences fell off considerably: in the second week, the samples at pH 4.5 released only 22% more CO_2 than controls and by the sixth week, the difference was down to 10% (Figure 1).

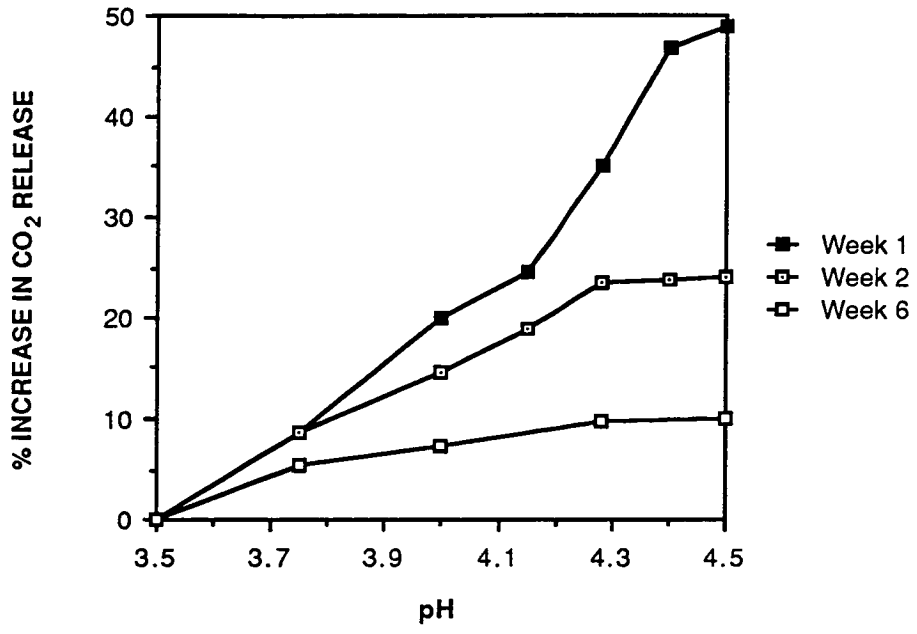


Figure 1. The affect of *small* pH increases induced by NaOH additions on CO_2 release rates during 6-week laboratory incubations. CO_2 release and pH values each based on three replicate subsamples. Control release rates: *week 1:* 5.7 µl CO_2 g^{-1} dw h^{-1}; *week 6:* 5.4 µl CO_2 g^{-1} dw h^{-1}.

Sample pH levels were measured again after six weeks, but had not changed from initial levels. The decreasing *variation* in treatment CO_2 release rates with successive weeks, despite stable pH levels, therefore indicated that the initial perturbation, perhaps associated with a pH-induced solubilisation of C compounds, had an effect over and above the pH increase *per se*.

Nitrogen release rates over the 42-day incubation period ranged from +7.2 µg N g^{-1} dw peat $week^{-1}$ to -2.6 µg N g^{-1} dw peat and were inversely related to C release: at the highest pH (pH 4.5), samples released the largest quantities of C but the lowest quantities of N while the reverse was the case at the lowest pH, *viz.* the controls (pH 3.5). Some additional samples included in this study were amended with equimolar quantities of KOH rather than NaOH. These showed that the identity of the cation (K^+, Na^+) was not important : C and N release rates were only related to measured changes in pH.

Mineral nutrients amendments. The addition of salts to acid peats at rates at or below field fertilization levels can induce significant reductions in pH levels as a result of H^+ ions being displaced from exchange sites into solution by the added cations (Figure 2). In the microcosm study, for example, the initial application of NH_4NO_3 to the NPK treatment was at a rate equivalent to 0.10 mmoles NH_4NO_3 g^{-1} dw peat, and when applied *alone*, it was found that this could induce a pH reduction of 0.22 pH units: from pH 3.50 to pH 3.28 (Figure 2). In the absence of pH controls, therefore, it is not posible to assess whether changes in CO_2 release are related to pH changes or to the identity of the ions in the salt. The studies outlined below, indicate that NH_4-N, PO_4-P, K, Ca and Mg have no positive effects *per se* on either CO_2 release or N release. Where there is an *apparent* positive effect, this can be traced to an increase in pH induced by the amendment.

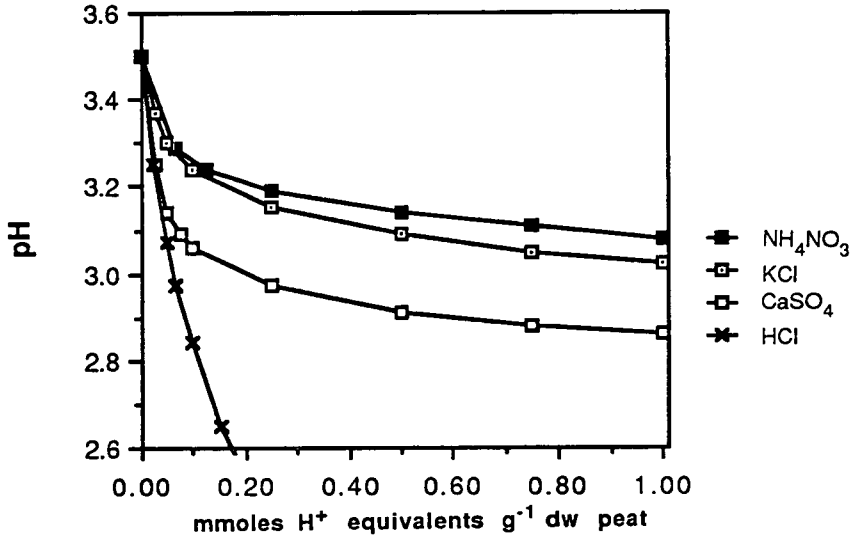


Figure 2. The effect of adding some salts on the pH of the acid oligotrophic peat used in these studies. pH was determined on three replicate subsamples (1:25 dw:water ratio) 2hrs after addition. Effects of HCl shown for comparison.

In the first study, Ca^{2+} and Mg^{2+} were applied to three sets of replicates as $CaSO_4$, $Ca(OH)_2$ and $Mg(OH)_2$ at a fixed rate of 0.10 mmoles g^{-1}. $CaSO_4$ had a negative

influence both on pH, which was reduced by 0.40 units to 3.10, and on CO_2 release rate, which was reduced by 28% in the first week of the incubation. This reduction in CO_2 release rate was still evident in the sixth week of the incubation (Figure 3).

When Ca^{2+} was added as $Ca(OH)_2$, pH was increased by 1.2 pH units and CO_2 release by 34%. Notably, this increase was not sustained, however, but declined, in a similar way to that observed for the NaOH treatment in the previous study (Figure 1): by the sixth week samples receiving $Ca(OH)_2$ released only 9% more CO_2 than controls. An important observation was that the $Mg(OH)_2$ treatment in this study had the same effect on pH and on CO_2 release as the $Ca(OH)_2$ treatment, indicating that *the identity of the cation is not important.*

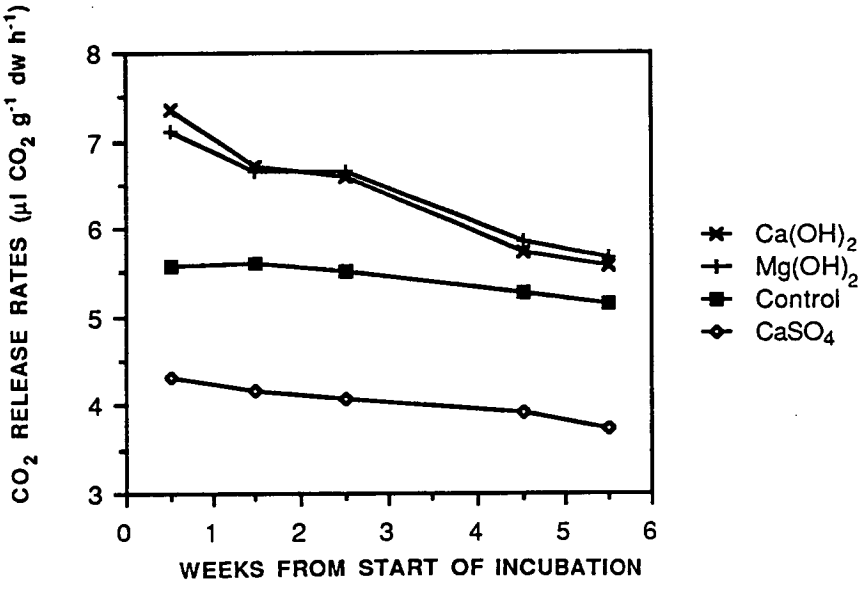


Figure 3. Effects of different compounds of calcium and magnesium on subsequent CO_2 release rates during 6-week laboratory incubations.

The second study reported here, was concerned with assessing the effects of N, P and K at the rates used in the microcosm study, on C release. To reiterate, these addition rates were calculated mainly on the basis of reported seedling uptake rates (Benzian and Smith, 1973). An additional treatment, number (3) below, was

included to assess the potential effects of the *pH changes* induced at these addition rates. The rates of mineral nutrient addition to treatments were equivalent to 0.10 mmoles NH_4NO_3, 0.016 mmoles K_2HPO_4 and 0.015 mmoles KCl, as indicated:

(1) Unfertilized controls.

(2) PK applied as a solution mixture of K_2HPO_4 and KCl.

(3) PK applied as a solution mixture of K_2HPO_4 and KOH.

(4) NPK applied as a solution mixture of K_2HPO_4, KCl and NH_4NO_3.

The impact of treatments on sample pH levels are shown in *Table 1*. Despite the small pH reduction induced by the NPK treatment, the influence on CO_2 release was profound: in the first week of incubations, this treatment reduced CO_2 release rates by 48% with respect to the control rate (Table 1). In subsequent weeks of the incubation, the negative influence of the NPK treatment increased in intensity, and by the sixth week NPK samples were releasing 54% less CO_2 than controls (Table 1).

Table 1. Effects of treatments (1) to (4) on pH and on subsequent CO_2 release rates during 6-week laboratory incubations. * Control CO_2 release rates: 5.9 (week 1) and 5.4 (week 6) $\mu l\ CO_2\ g^{-1}$ dw peat. All fertilized treatments significantly different from unfertilized control ($p<0.01$).

Treatment	pH	CO_2 release (week 1)	CO_2 release (week 6)
(1)	3.50	-*	-*
(2)	3.58	- 20%	- 21%
(3)	3.78	+ 7%	+ 4%
(4)	3.36	- 48%	- 54%

A further departure from the close association between induced pH changes and CO_2 release rates which had come to be expected on the basis of the previous studies, occurred with the K_2HPO_4/KCl treatment. While this induced an *increase* in pH, it

had a negative effect on CO_2 release (Table 1). This contrasted with the K_2HPO_4/KOH treatment which increased CO_2 release by 7%, an amount which could have been expected (e.g. see Figure1) from its increase on pH (Table 1). It is possible that the negative effect of the K_2HPO_4/KCl is related to the presence of Cl^-, but on the basis of these results it is only possible to speculate about this.

The very large negative effect of the NPK treatment on CO_2 release appears to be related to the presence of NH_4NO_3. As noted above, this negative effect is very much greater than would occur in response to the measured pH reduction and this raises the possibility that the identity of the ions (NH_4 or NO_3) may be important in repressing microbial activity. NH_4-induced repression of ligninolytic activity has already been reported (Keyser *et al.*, 1978; Kirk *et al.*, 1978; Kirk and Fenn, 1982) and it is possible that other fungal enzyme systems are also repressed. Further studies, using NH_4^+ salts at various addition rates, and in combination with pH regulating components would need to be undertaken in order to ascertain if this is the case. The possibility that the reduction in release is related to the presence of NO_3^- cannot yet be ruled out.

To summarise: the results of both of the above studies when taken together, support the view reached on the basis of the **pH adjustment** studies, that the effect of metallic cations - K^+, Na^+, Ca^{2+}, and Mg^{2+} - on CO_2 release is only related to their effect on pH: these cations make no positive contribution to decomposition rate (CO_2 release rate). The results of the second study have shown, however, that other ions have an effect on CO_2 release over and above what would be expected from the changes induced on pH. An NPK treatment, for example, which reduced pH by just 0.14 pH unit reduced CO_2 release by 48% (Table 1). A much greater reduction in pH (0.40 pH units), induced by a $CaSO_4$ amendment reduced CO_2 release by a much smaller amount (by 28%, Figure 3). The major reduction in CO_2 resulting from NPK fertilization appears to be associated with the presence of NH_4NO_3. In the light of these results, the view that the decomposition of peats is constrained by low amounts

of available nutrients cannot be upheld.

Labile carbon amendments. The addition of labile C supplements to soils results in immediate and marked increases in CO_2 release. As discussed previously, however (Chapters 1, 3 and 5), reports of subsequent positive effects on mineral N release are very uncommon. The series of experiments reported below were designed to test the effects of glucose amendments with and without mineral nutrient amendments on subsequent C and N release during incubations.

In the first glucose-amendment study, glucose was added at the rate of 25 mg g^{-1} dw peat to four treatments. These treatments were identical to those used in the previous section and to reiterate, the mineral nutrient application rates were the same as those used in the microcosm experiment (Chapter 3):

(1) Control (unamended)	**+ 25 mg glucose g^{-1}**
(2) K_2HPO_4/KCl	**+ 25 mg glucose g^{-1}**
(3) K_2HPO_4/KOH	**+ 25 mg glucose g^{-1}**
(4) K_2HPO_4/KCl/NH_4NO_3	**+ 25 mg glucose g^{-1}**

Four additional treatments receiving no glucose were run concurrently.

Glucose was added to samples as a aqueous solution at a concentration of 25 mg glucose ml^{-1} and samples were then incubated (84% moisture; 20°C). Over the first week of a 6-week incubation, the amount of CO_2 released into headspace was measured daily. A departure from usual procedures was as follows: after daily measurement bottle-caps and seals were removed and headspace was ventilated for 2 *minutes only* using a flow of compressed air. Bottles were then resealed. Headspace sampling after resealing confirmed that headspace CO_2 levels had been reduced to ambient levels. This procedure was adopted in order to maximise the number of sampling periods during the initial period after glucose amendment, a period when large and rapid changes in CO_2 release rates could be anticipated (see Figure 4). After one week, additional replicate samples from glucose amended treatments were extracted in order to determine whether any glucose had not been consumed, but

amounts measured were less than 1% of the amounts applied.

CO_2 release rates were as shown in *Figure 4*. The percentage of the glucose amendment equivalent to the excess release of CO_2 was calculated for each treatment by deduction of release rates in corresponding unamended (no-glucose) treatments. The accumulated percentages over the incubation period are shown in *Figure 5*.

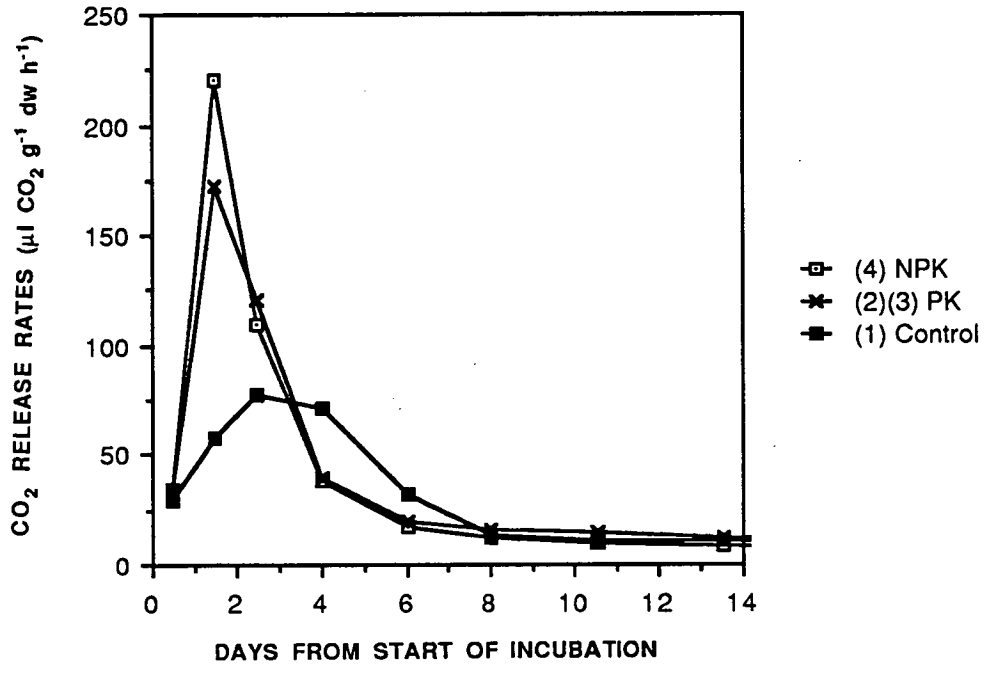


Figure 4. CO2 release rates after a glucose amendment of 25 µg g^{-1} dw peat. Release rates in corresponding no-glucose treatments were similar to those listed in *Table 1*. Values for both PK treatments were the same.

Results indicate that NPK and less notably PK enhances the recycling rate of carbon from a labile amendment: after 21 days 52% of added glucose still remained in unfertilized controls, compared with only 38 and 29% in PK and NPK fertilized controls respectively. Corresponding values after 42 days were 46%, 31% and 25% (Figure 5). At this stage, CO_2 release rates were almost at the levels of corresponding no-glucose treatments. For example, at 42 days, the glucose amended NPK treatment was releasing CO_2 at a rate of less than 1 µl CO_2 g^{-1} h^{-1} in excess of the corresponding no-glucose treatment and this differential appeared to be decreasing with successive measurements. Even if a differential of 1 µl CO_2 g^{-1} h^{-1} was sustained it would be a

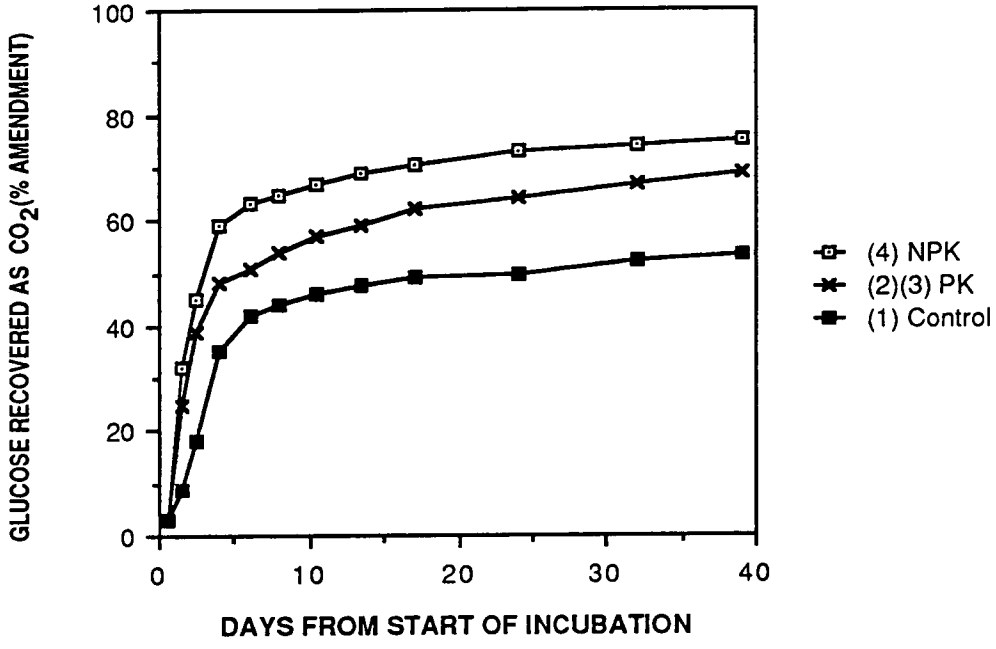


Figure 5. Glucose recovered as CO_2 as calculated from glucose-amended minus unamended release rates. Values for both PK treatments were the same ±1%.

further 195 days before the 6.24 mg glucose g^{-1} remaining from the 25 mg glucose g^{-1} amendment could be accounted for as excess CO_2 release. Notably, the release of CO_2 from both glucose amended PK-fertilized treatments was the same. This contrasted greatly to the differential effects of these treatments on release from unamended peats (Table 1).

The implications of these results are that a plentiful supply of mineral nutrients enhances the decomposition rate of labile substrates. This may relate to a lower substrate-to-biomass conversion efficiency of microorganisms exploiting highly favourable substrate conditions, as discussed by Heal and Ineson (1984). In the knowledge that all glucose was consumed in the first week of incubations (as noted earlier) another major implication of these results is that in the absence of mineral nutrients, more labile C is committed to a refractory pool of C. The irony of these studies, therefore, is that they provide no evidence of a priming effect of labile C on the decomposition of peats while they open up the possibility that labile C actually contributes to the formation of the refractory products of microbial synthesis activities. Wagner (1968) reported that 37% of ^{14}C remaining in soil following a ^{14}C-labelled

glucose amendment was present in the humus fraction after 150 days. This percentage compares favourably with the values reported here - 25% (NPK) to 46% (unfertilized) at 42 days - given that the glucose remaining in the peat at this stage of the incubation was being released at such a slow rate (Figure 5).

To conclude, while the presence of a plentiful supply of mineral N, P and K may have no positive influence on the decomposition of *refractory substrates*, as shown earlier for an acid oligotrophic peat, it greatly enhances the decomposition of a *labile carbon amendment.* In the absence of a plentiful supply of mineral N, P and K a a much higher proportion of a labile carbon amendment will become trapped in the pool of refractory substrate residues.

The second glucose-amendment study was designed to look specifically at the influence of glucose on mineral N dynamics. Six sets of 1.5 g dw subsamples which had received no fertilizer were amended with glucose at the following rates:

0 1.0 2.5 5 12.5 25 *mg glucose g^{-1} dw peat*

and then incubated at 84% moisture and 20°C for six weeks. Release rates of N over the incubation period ranged between +8.8 μg g^{-1} $week^{-1}$ and -28.0 μg g^{-1} $week^{-1}$ as shown in *Figure 6*. At an amendment rate of between 2.5 and 5 mg glucose g^{-1} there was a transition between net release and net immobilisation of N.

These results show that at least in the short term (6 weeks), glucose amendments greatly reduce the availability of mineral N. It is important to remember that it is possible that single applications of glucose result in explosive population growth and that those microorganisms which benefit most from the amendment may contribute least to N release activity.

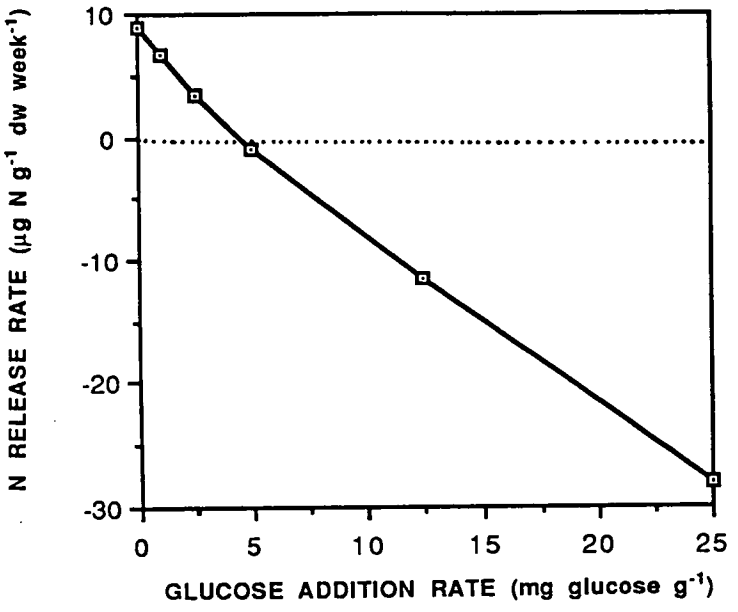


Figure 6. Mineral N release rates during six-week incubations (84% moisture; 20°C) following glucose amendments at increasing addition rates. Mineral N concentration at start of incubation: ***510 µg g^{-1} dw peat.***

CHAPTER 7

(THESIS DISCUSSION AND CONCLUSIONS).

INFLUENCE OF PLANTS ON C AND N RELEASE FROM ACID OLIGOTROPHIC PEATS.

GENERAL COMMENTS.

CO_2 release from de-rooted peats during incubations: a sensitive index of labile C availability to microbes?

All the incubation studies relating to de-rooted peats (chapters 3, 4, 5) have shown that there is no significant correlation, either positive or negative, between C release and N release within the time-scale of incubations (usually 42 or 60 days). A prospective reason for the absence of a significant relationship is that substrates comprise a mixture of C- and N-fractions of varying lability and that some conditions can influence the mineralization rates of C fractions without having a related influence on the mineralization of N fractions. This thesis envisages that provided mineral nutrients are not limiting for microbes - which appears to be the case in acid peats - then *carbon* mineralization rates depend largely on the amounts of assimilable C constituents in a substrate while *nitrogen* mineralization rates depend not just on the amounts of humus-N constituents but also on the prevalence of additional conditions which favour their breakdown by enzymes.

Against this background, the amounts of CO_2 released from heterogeneous substrates within the time-scale of incubations appear to be of limited value for understanding the conditions which favour mineral N release. As illustrated in the abiotic studies, however, C release rates are highly sensitive to changes in substrate conditions, probably because these readily influence amounts of labile C constituents. This means CO_2 release studies can provide a detailed insight into the conditions which favour increased rates of decomposition. While this thesis stresses the potentially autonomous nature of mineral-N dynamics with respect to C dynamics within an arbitary time-span, any influence which increases CO_2 release rates may in the long-term have a positive effect on mineral-N release rates. It is often reported, for example, that the positive effects of lime additions to oligotrophic peats on N release

become apparent several years after application. A possible explanation for this is that lime-induced solubilization of C results in increased C availability; and that an enhanced labile C economy results in a faster rate of humification and a faster turnover of N-retaining humus constituents.

Mineral-N release from *de-rooted peats* during incubations: no index of N availability to plants ?

The microcosm study showed that N release rates from unamended de-rooted peat samples over 6-week incubation periods did not reflect N uptake rates by plants. Similar incubation studies involving the greenhouse peat identified *disturbance* as the likely impulse to N release, and showed that no release occurred in the absence of disturbance. It is not yet possible to indicate precisely what aspect of disturbance provides the impulse to release. However, both studies cast serious doubt on the value of routine incubations of *peat* samples for the purposes of assessing N availability to plants.

A particular recommendation which emerges from these results is that the influence of different planting regimes on microbial processes seems more likely to reflected in the measurements of particular enzymatic activities in peats, than in N release rates during incubations, conducted, as in this research, up to many weeks after live roots have been removed. The plants themselves must remain the best indicators of *absolute* nutrient release rate. All the results from the incubations of de-rooted planted peats have shown that CO_2 release rates are highest at the outset of incubations and that they rapidly diminish over subsequent weeks. This indicates that assay procedures aimed at assessing plant-related influences on specific enzymatic activities need to be applied as close to the time of sampling and de-rooting as possible. The study in the microcosm experiment showing that *N release from an amino-N amendment* was closely correlated to *N uptake rates*, indicates that an approach such as this may provide a relative measure of *in situ* release in comparative studies involving different trees or different peats.

Finally, as for the mixture effect, the differences in N uptake between pine and spruce in the microcosm study suggests that it may be the presence of pine or its mycorrhizal fungi, rather than a mutualistic interaction between species, that may be responsible for this. If indeed this is the case, then the enhanced N nutrition of spruce in the presence of pine is probably a secondary effect, somehow associated with the overall

increase in stand N capital attributable to pine. Future experimental research aimed at determining a clearer mechanistic understanding of the benefits to spruce in mixtures may best be focussed therefore on developing a clearer understanding of individual species grown in pure plots on oligotrophic peats.

CONCLUSIONS.

In conclusion, taking into account the results of all the studies reported here, it is opportune to recall the four questions that were posed at the outset of this thesis, and to consider whether they can now be answered.

Do different tree species take up different amounts of N from the same volume of peat ?

In **the microcosm study**, uptake of N by pine was shown to be 39% higher than uptake by spruce. It was acknowledged that these differences may relate more to species-related *mycorrhizal* characteristics under the conditions of the growth room, than to the tree species themselves. Unfortunately the experimental design did not favour additional quantitative studies of mycorrhizal development. Both species in this study came from the same nursery source and both were mycorrhizal at planting, however, general observations throughout the experiment and at harvesting indicated that mycorrhizal infection was much greater in pine than in spruce roots. As discussed in detail, this appeared to be reflected in higher amounts of humic-like substances, possibly fungal melanins, in acetic acid extracts of peats under pine than of those under spruce. These observations are in themselves of interest, and leave open the possibility that spruce also has less effective mycorrhizal associations in oligotrophic peats *in the field.* Nevertheless, a lesson to be learnt from this study is that future experimental research needs to take account of quantitative and qualitative differences in mycorrhizal infection in addition to the tree species themselves. Notably, the amounts of mineral N remaining in the peats under pine and spruce at harvesting were quantitatively insignificant and this observation might suggest that if mycorrhizal fungi *do* account for differences in uptake, then the success of an effective mycorhizosphere is not simply related to spatial exploitation of substrate as is often suggested.

Do some tree species contribute more soluble C to peats than others?

It should be noted that this research did not attempt to quantify the inputs of soluble C associated with the *litters* of different species: though it did attempt to put the concentrations of soluble C found in different forest floor horizons into quantitative

context, it was mainly concerned with looking for differences in the soluble C levels that could be associated with the *roots* of different species. The results indicate that potentially large species-related differences may exist: **the microcosm study** was able to show that there were substantially greater quantities of soluble carbohydrate, as measured by anthrone, in de-rooted peats under pine than in de-rooted peats under spruce. Notably, some reservations were expressed about these results, *firstly*, because it was observed that there were generally greater quantities of dissolved humic material in extracts of peats under pine than in those under spruce, and it was considered possible that the intense hydrolytic conditions involved in the reaction could result in some solubilisation of carbohydrate that was bound up in fungal melanin or humic components; *and secondly*, because *free glucose concentrations* (measured enzymatically) were not significantly different in the two treatments and did not provide what would have been strong supporting evidence therefore, of a more lucrative labile C economy under pine than under spruce. **The greenhouse study** was able to show, however, that *there definitely are* large differences in carbohydrate levels that can be associated with the roots of different species. Here, *both* mean soluble carbohydrate levels (measured by anthrone) *and* mean free glucose levels (measured enzymatically) were many times higher in de-rooted peats under birch than in those under spruce, pine or larch.

To return to the question, however, neither **the microcosm study** nor **the greenhouse study** have been able to show that trees are definitely the source of the carbohydrate: it is not been determined whether plants influence soluble carbohydrate levels in peats *directly*, by releasing carbohydrate from their roots, or *indirectly*, by producing more favourable conditions for the enzymatic release from native organic matter.

Do levels of soluble C relate to CO_2 release rates?

Most studies reported here indicate that a good relationship usually exists between a substrate's carbohydrate concentration and its CO_2 release rate. This relationship appears to exist on two levels of observation, a broad level, and a finer level:

(1) A broad level of observation. **The field study** showed that when substrates of widely different character are compared (e.g. L *vs*.FH *vs*. peat), CO_2 release rates are reasonably well correlated to soluble carbohydrate or free glucose levels ($p<0.01$). In such a broad comparison, soluble C levels might well reflect intrinsic substrate quality: for example, deteriorating quality - as

indicated by CO_2 release rate - in the sequence *fresh litter>old litter>FH>peat* was generally found to be reflected in a step-wise reduction in soluble carbohydrate and free glucose levels.

(2) A fine level of observation. Both **the microcosm study** and **the field study** showed that among substrates which in all respects appear similar, close correlations can usually be demonstrated between soluble carbohydrate concentrations and initial CO_2 release rates. At this level of observation - where the substrates appear to be identical - soluble carbohydrate or free glucose levels appear less likely to be indicators of *intrinsic* substrate quality than of factors associated with plants, *viz.* direct inputs of carbohydrate from roots *or* indirect influences leading to the solubilisation of native organic matter, as discussed above.

To summarise, the concentrations of soluble C in field substrates usually relate well to CO_2 release rates. However, in peats under the influence of live roots, it is not clear whether increases in CO_2 release rates are related simply to the decomposition of carbohydrate released from plant roots or to enhanced decomposition rate of native organic matter.

Do levels of soluble C relate to mineral N release rates?

In all of the incubations of de-rooted peats, no evidence emerged of a relation between carbohydrate levels at the start of incubations and subsequent N release rates (chapters 3, 4 and 5). Given that these incubations were conducted in the absence of living plant roots, however, the results cannot be taken to suggest the absence of an *in situ* relationship.. Evidence for an *in situ* relationship can be found in **the microcosm study**, which showed a high correlation between plant N uptake values in PK-fertilized tubs and soluble carbohydrate concentrations ($n=15$; $r=0.79$; $p<0.01$). Also in **the microcosm study**, when samples were amended with an amino-N amendment, a close association between mineral N release rate and carbohydrate concentration was reported ($n=15$; $r=0.79$; $p<0.01$). This indicates that under certain conditions a relationship does exist, though it is not of course clear whether it is a mechanistic one.

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